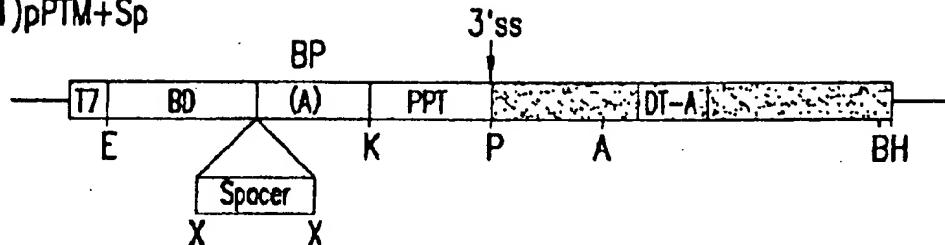


FIG. 1A

(1)pPTM+Sp



(2)pPTM+Sp

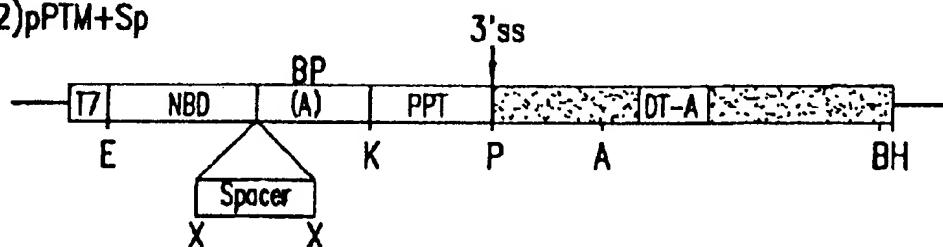


FIG.1B

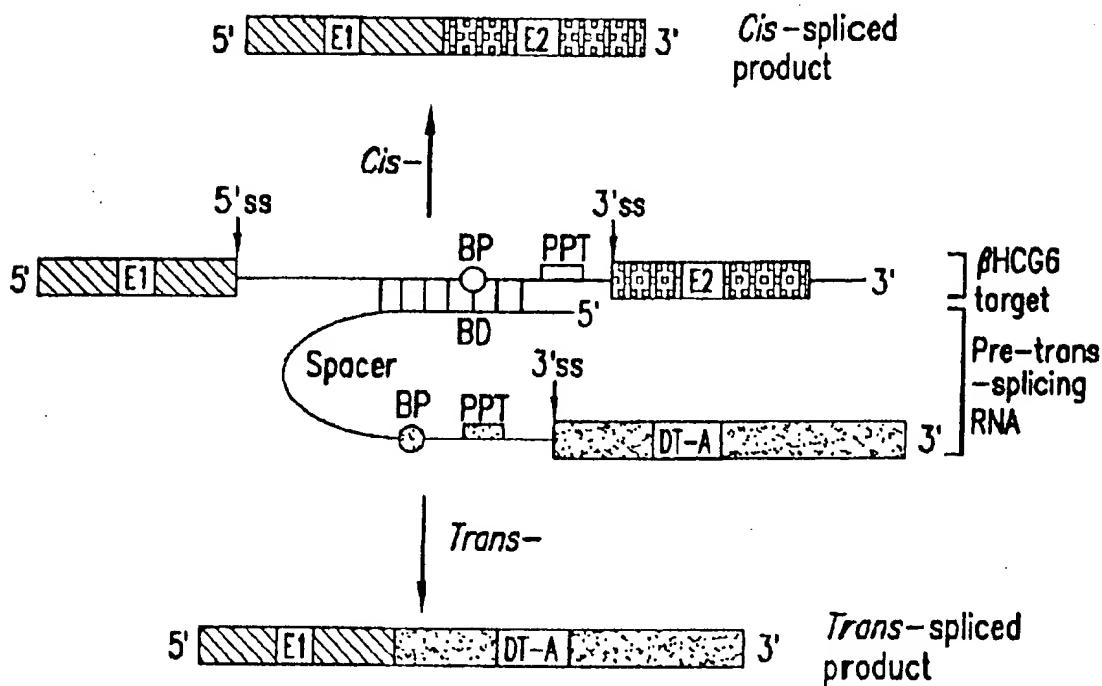


FIG.1C

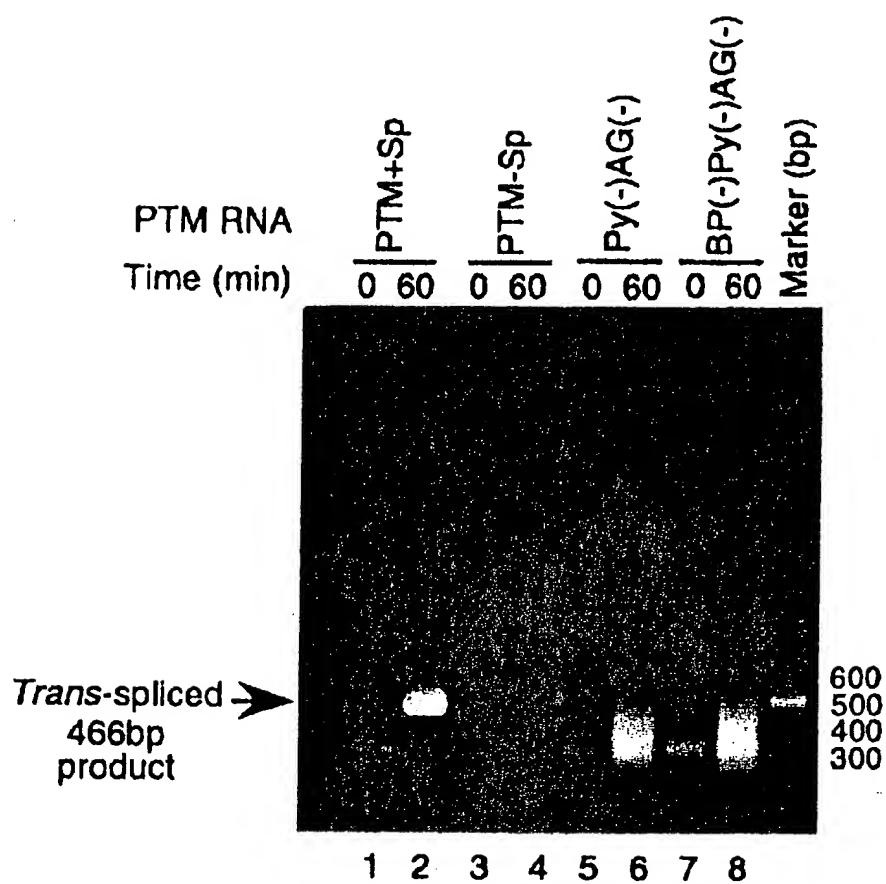


FIG. 2A

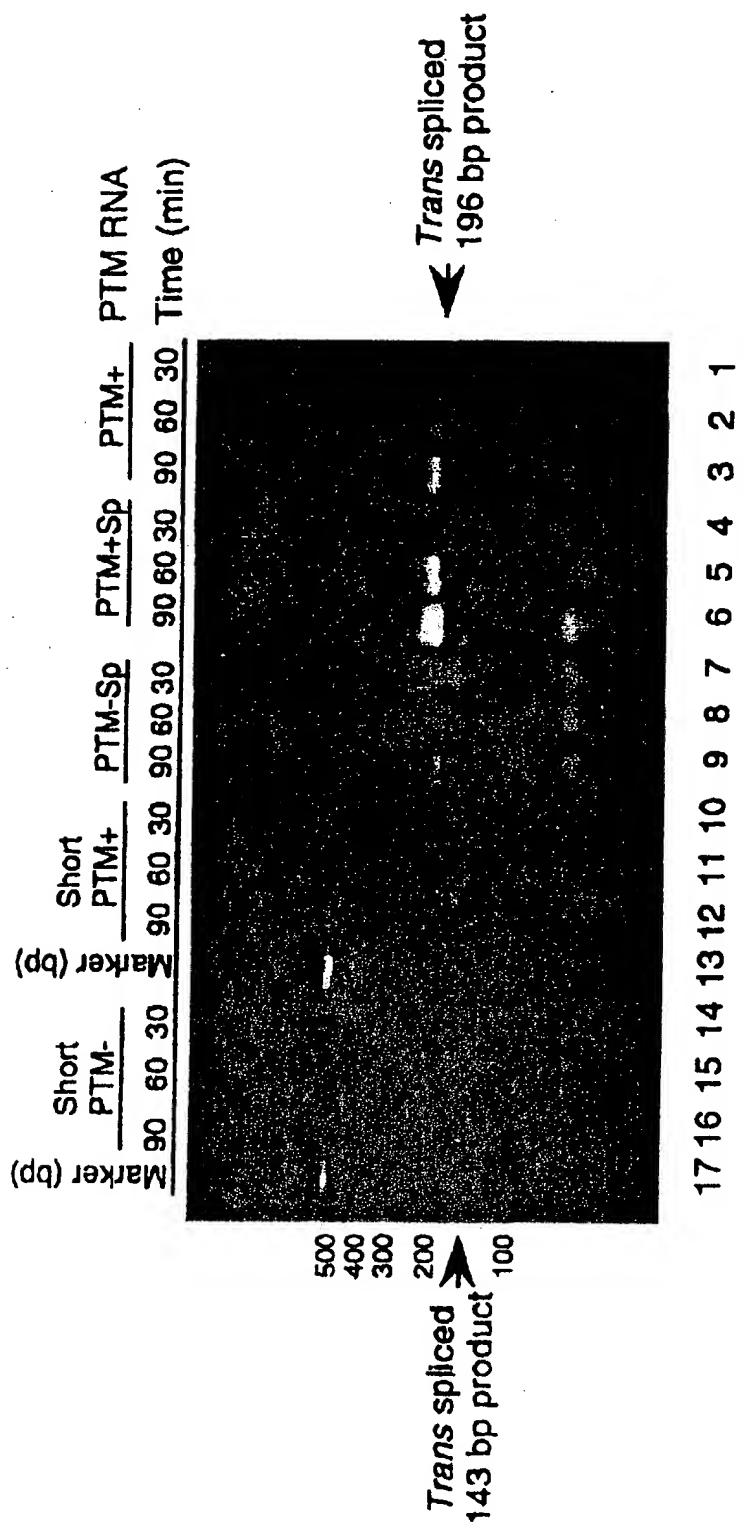


FIG.2B

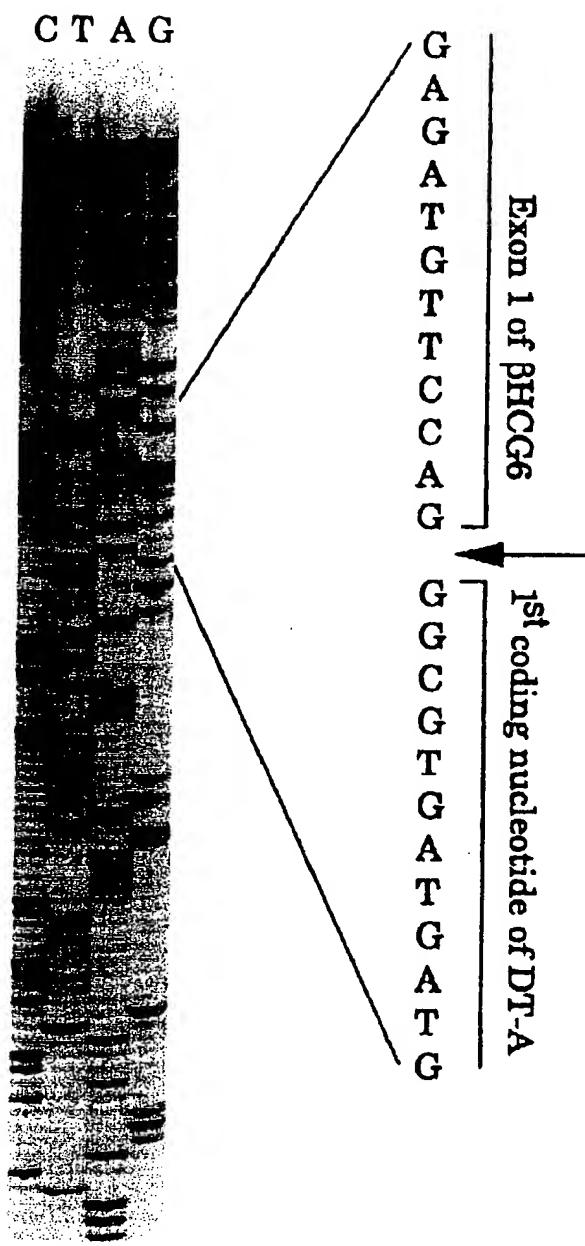
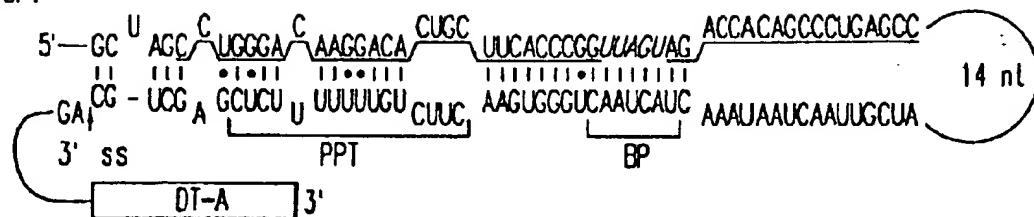
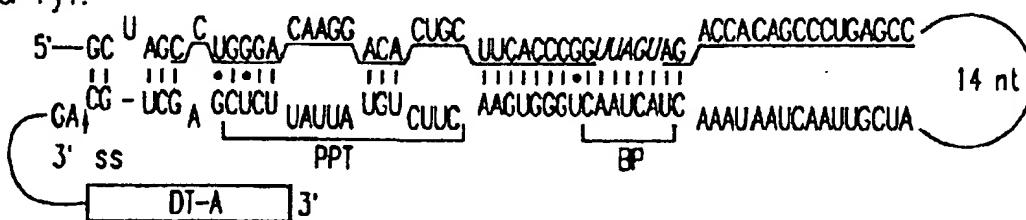


FIG.3

## 1. PTM+SF:



## 2. PTM+SF-Py1:



### 3. PTM+SF-Py2:

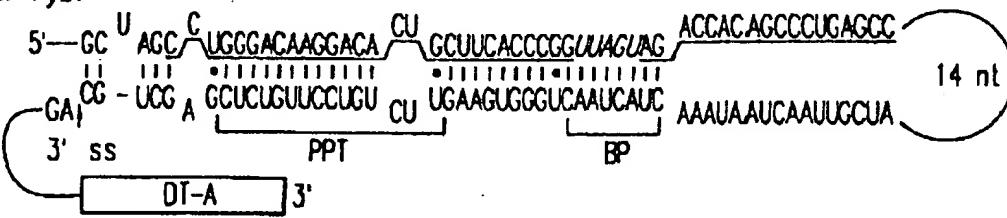


FIG. 4A

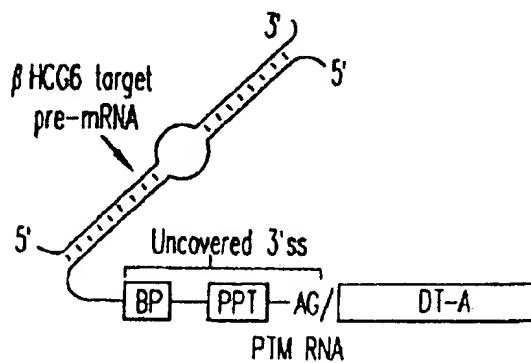


FIG. 4B

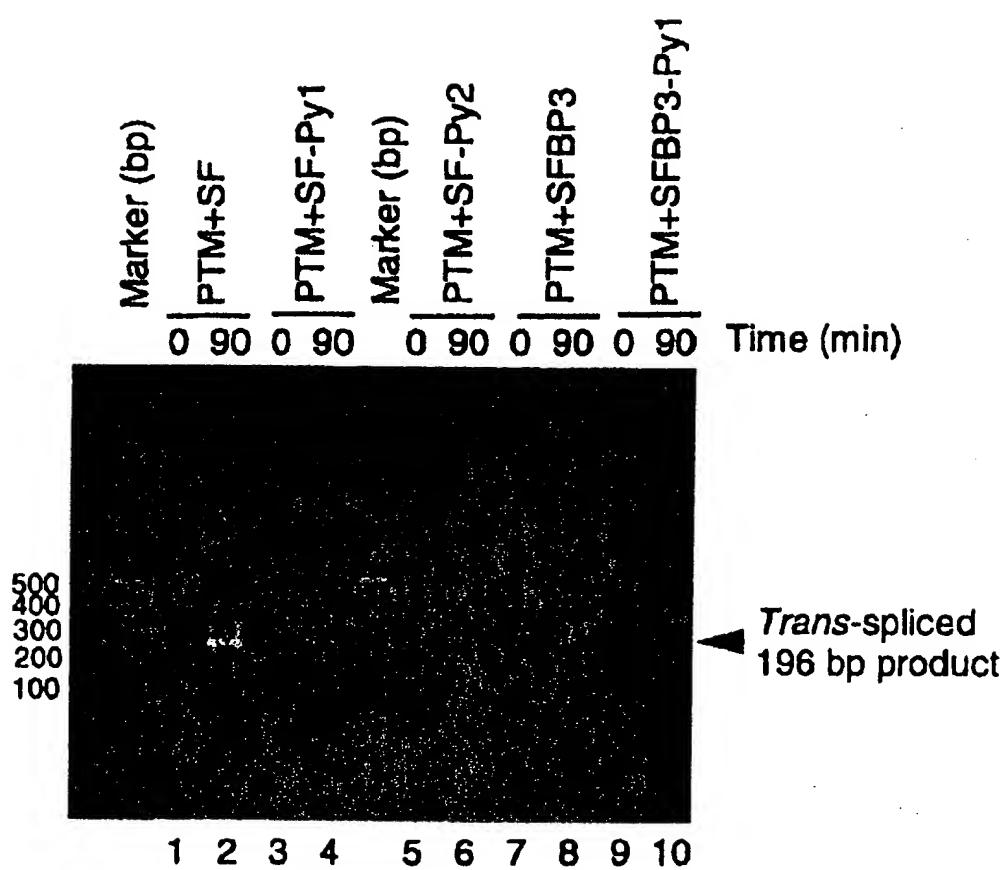


FIG.4C

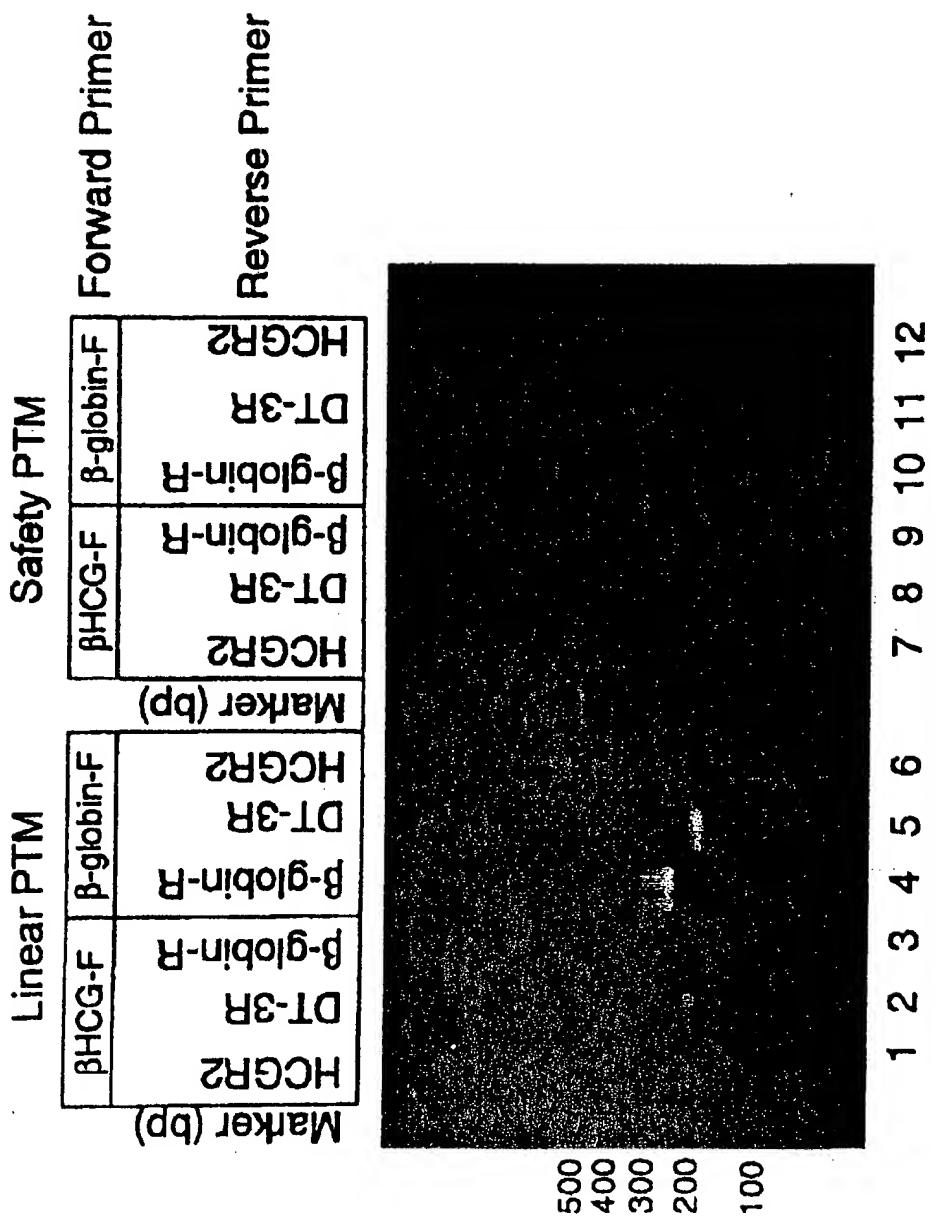


FIG.5

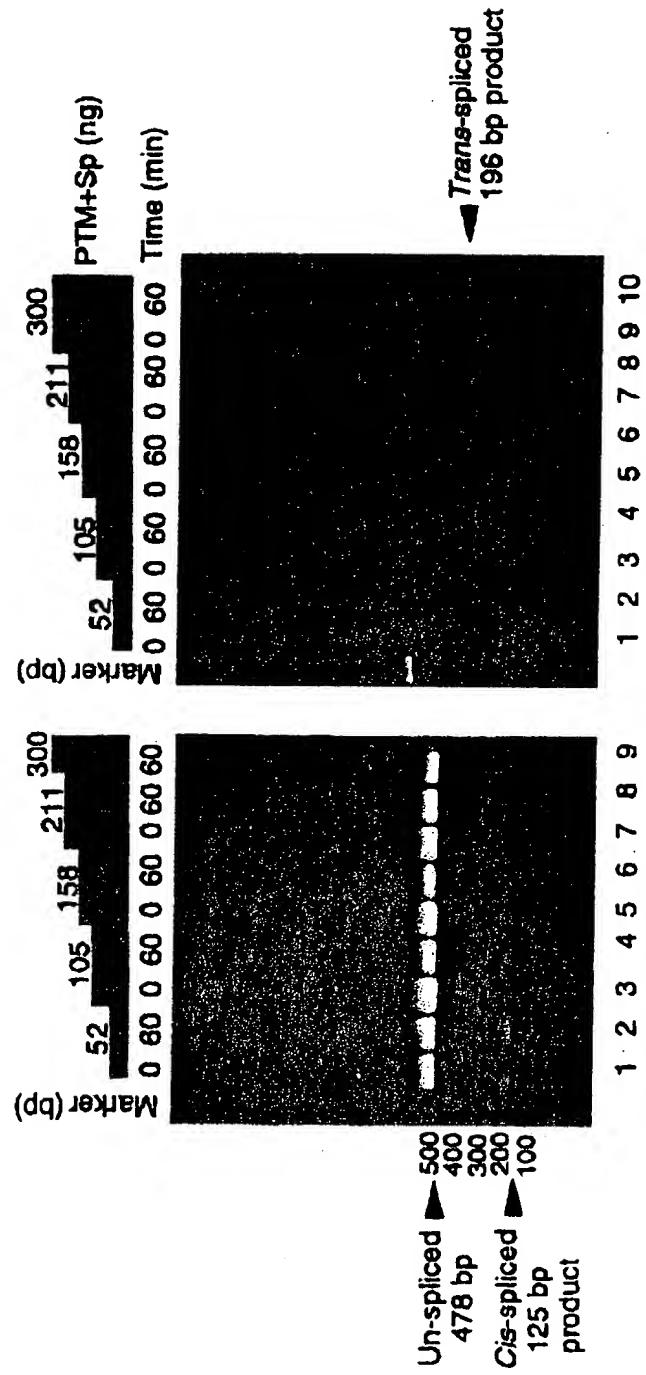


FIG. 6A

FIG. 6B

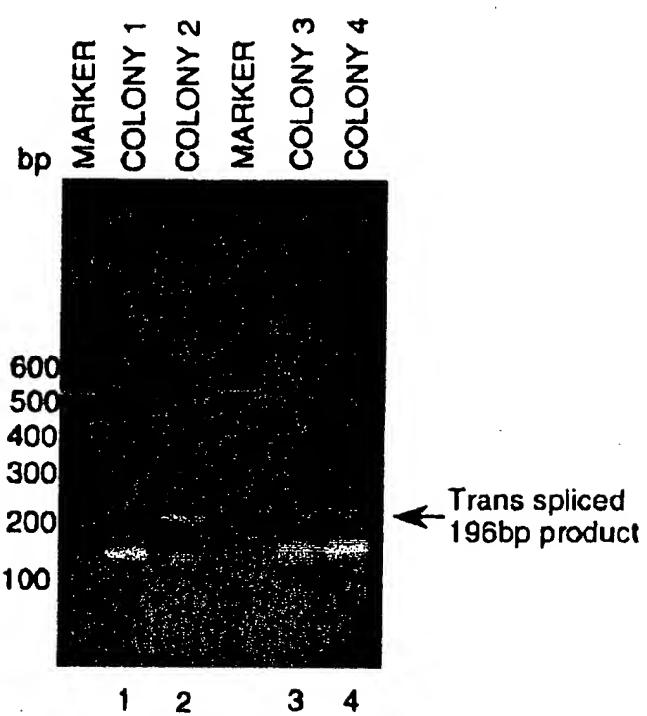
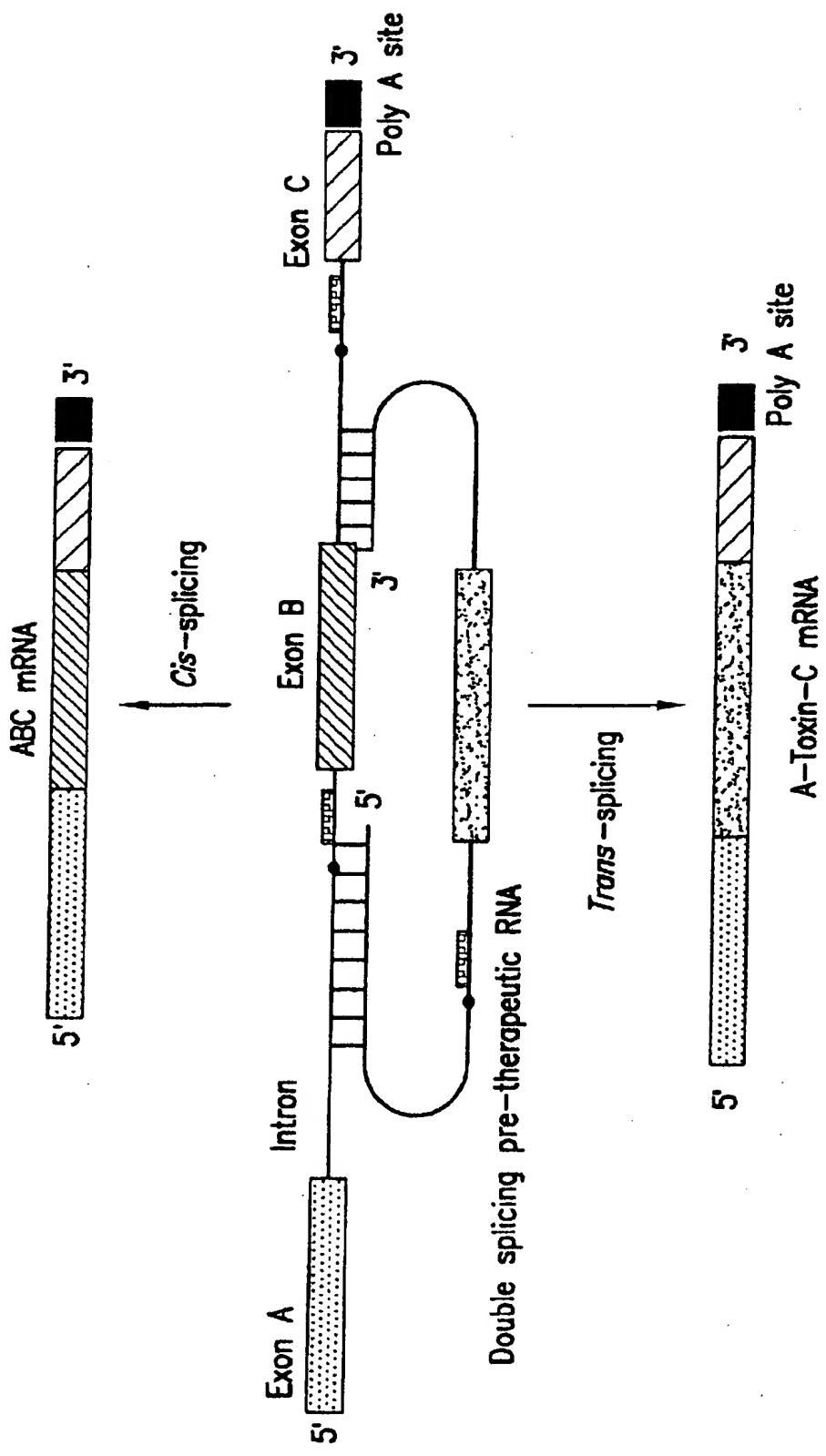


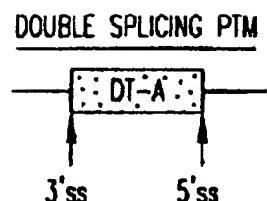
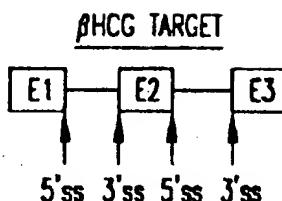
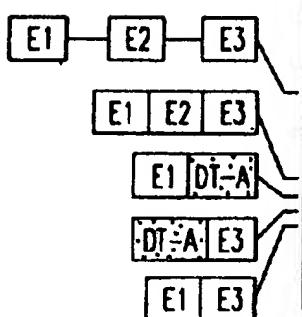
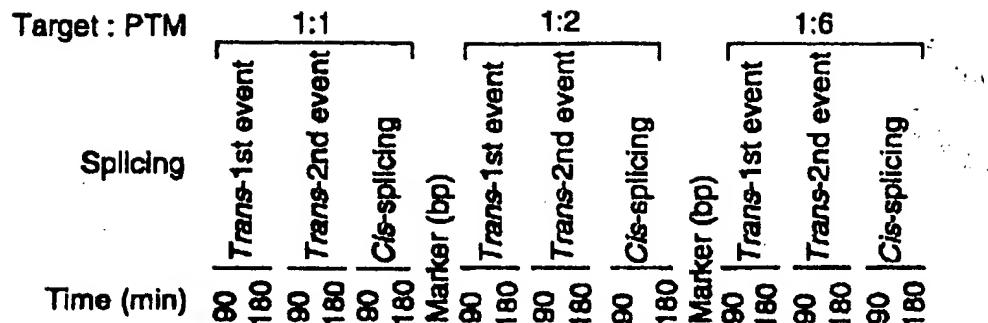
FIG.7A

EXON 1 OF  $\beta$ HOOG6 |  
5'-CAGGGACCCAAAGGATGGAGATGTTCCAG-CCGGCTGATGATGTTGTT  
| 1ST CODING NUCLEOTIDE OF DT-A  
GATTCTCTTAAATCTTTGTGATGGAAAACTTTCTTCTGTAACCAACGGACTA  
AACCTGGTTATGATTCATTCAAAA-3'

FIG. 7B



**FIG. 8A**



### *Cis*-spliced products

E1 E2 E3 = NORMAL *cis*-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

### Trans-spliced products

E1 [DT-A] = 1st EVENT, 196bp. *Trans-SPLICING* BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. Trans- SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

**FIG. 8B**

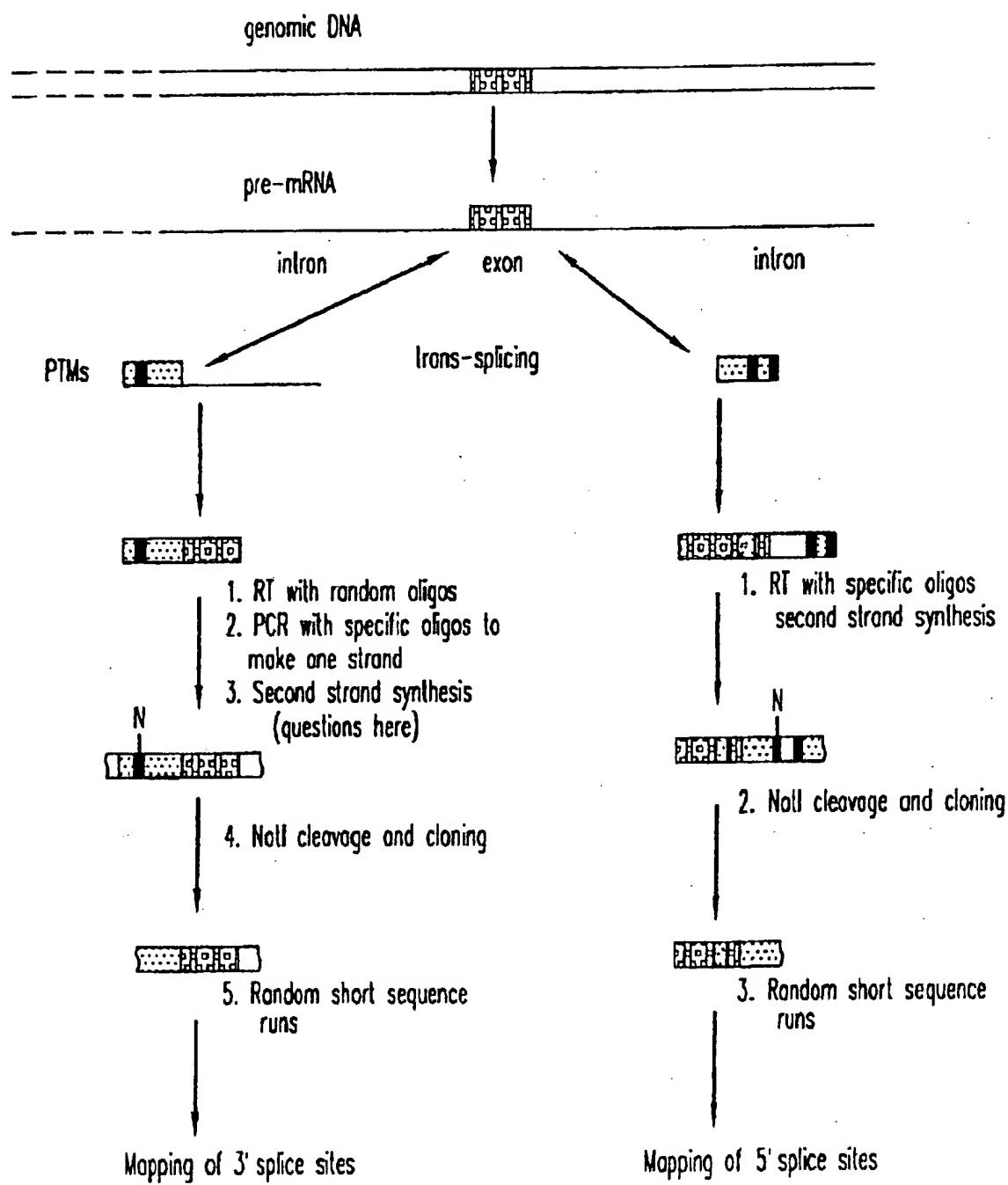
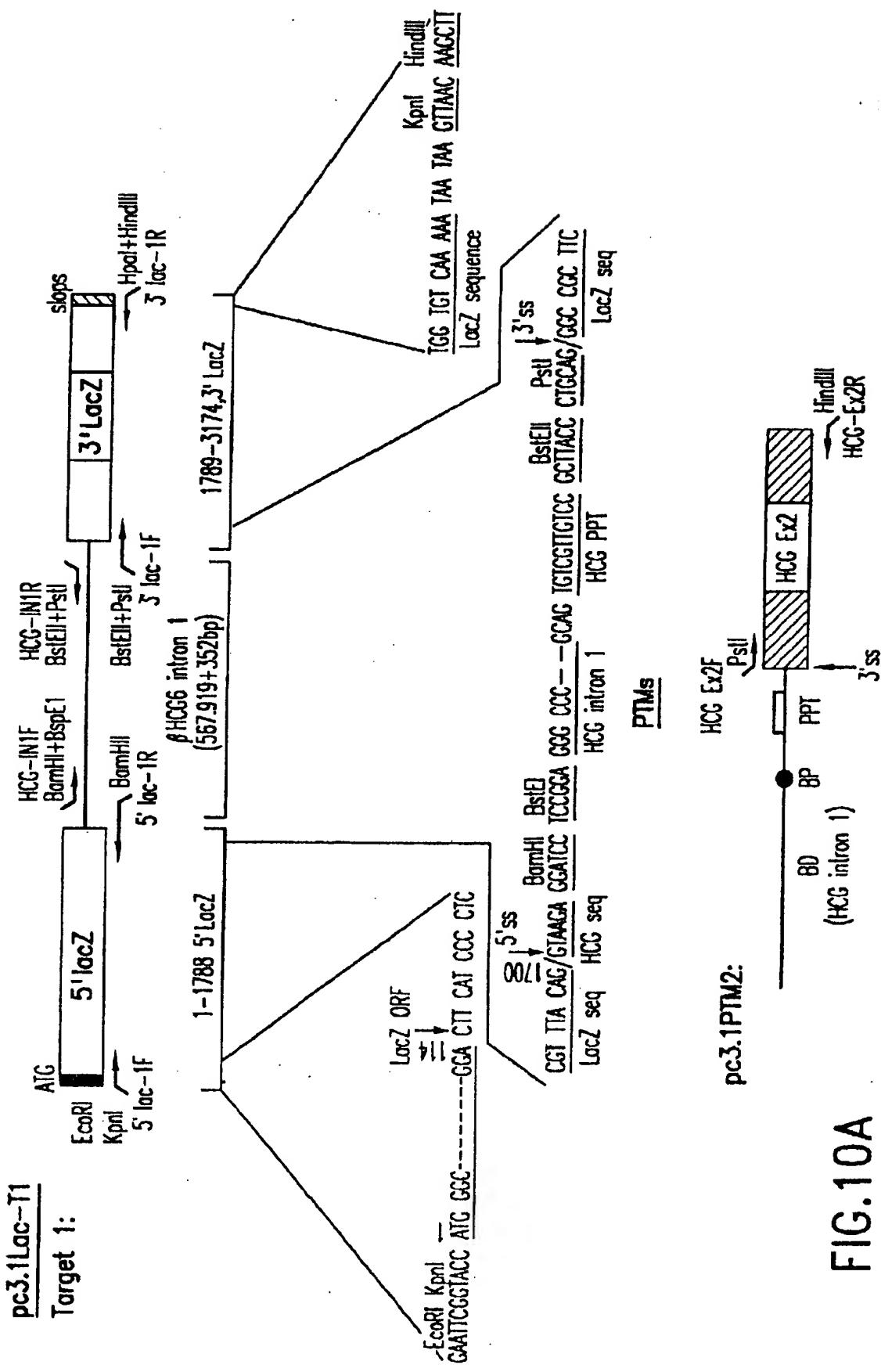


FIG.9



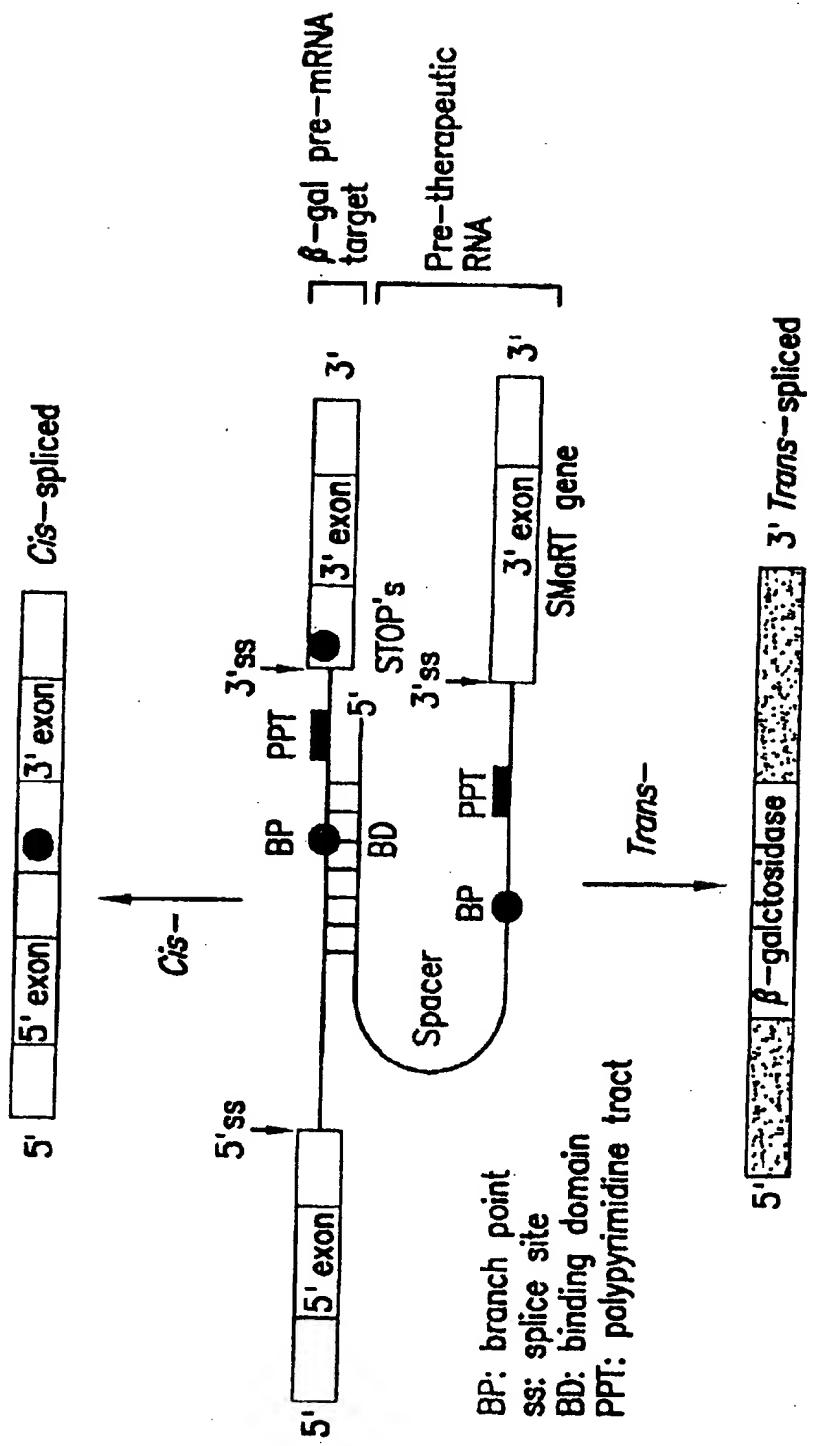


FIG. 10B

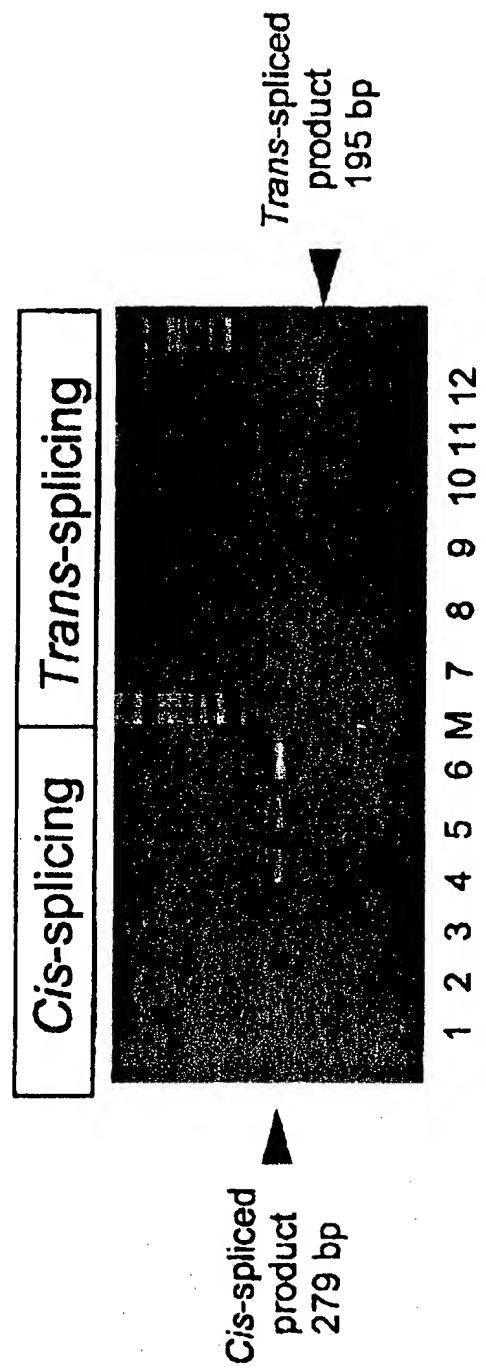
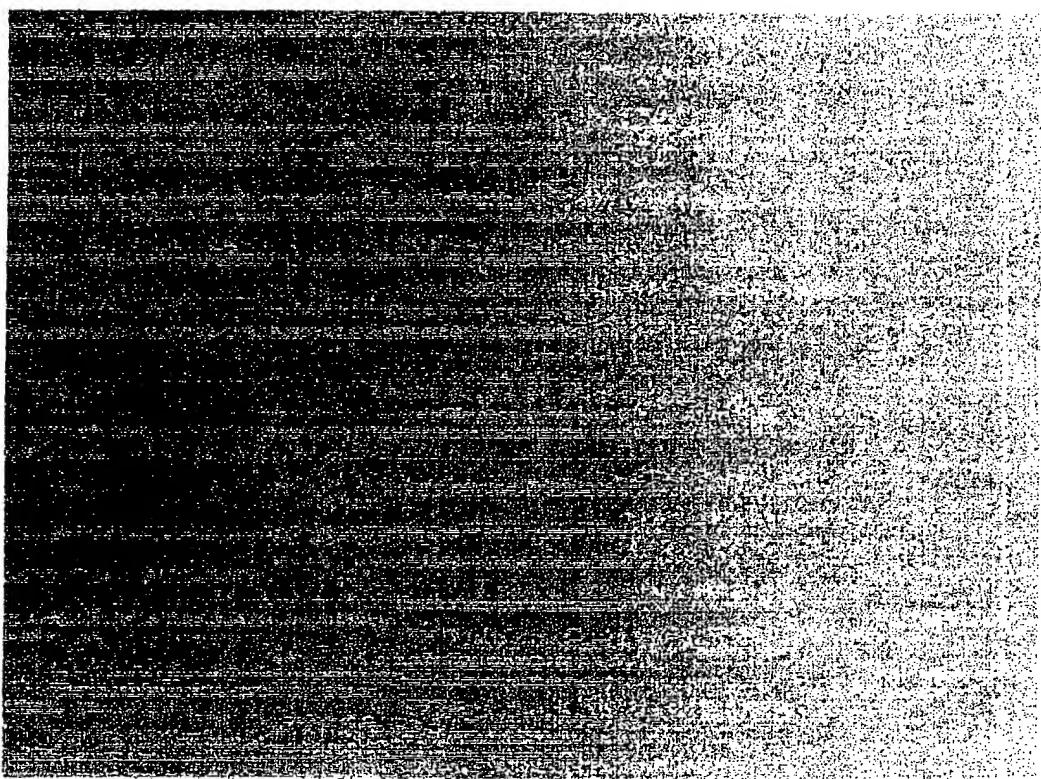
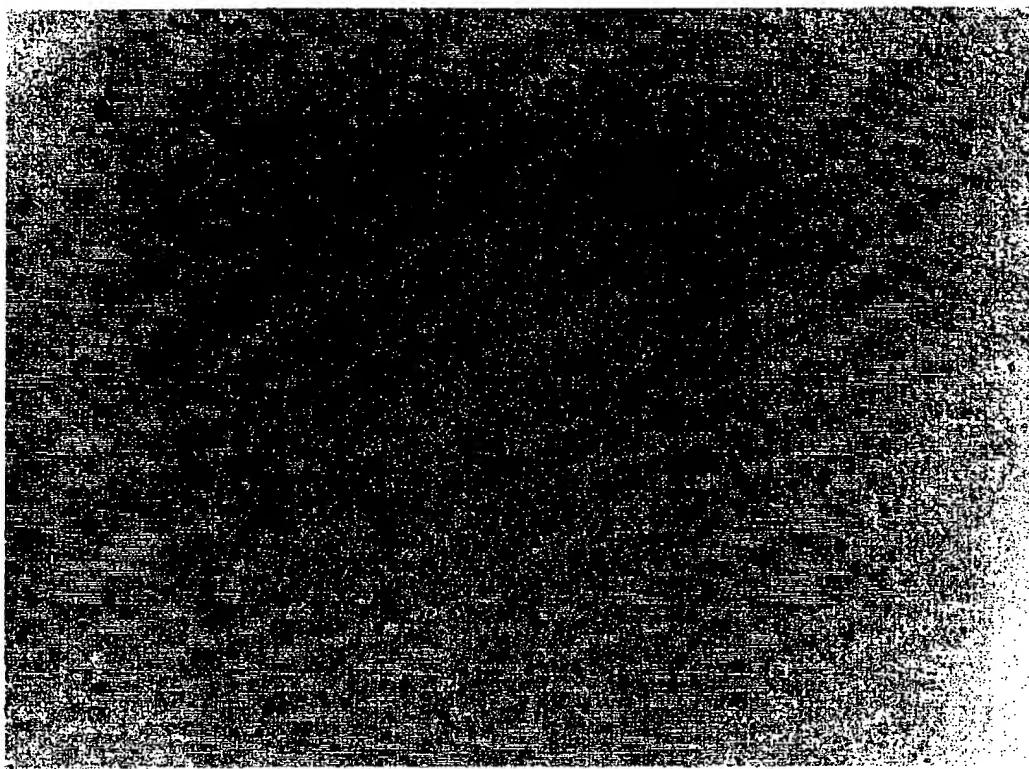


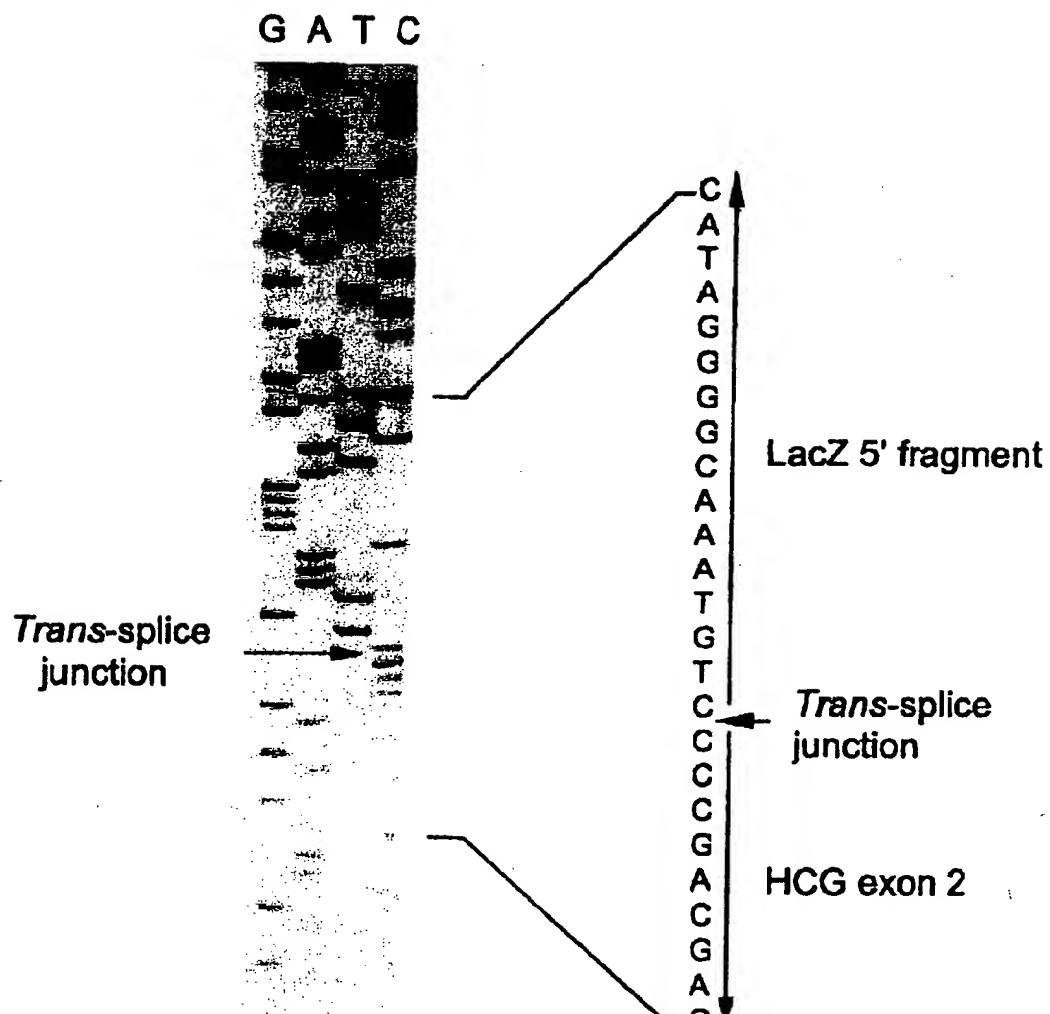
FIG. 11A



**FIG.11B**



**FIG.11C**



**FIG.12A**

1. NUCLEOTIDE SEQUENCES OF THE *cis*-SPLICED PRODUCT (285 bp):

BioLac-TR1

GGCTTCTACCTGGAGACGGCGCCGCTGATCCTTGGAAATACGGCCAGGGATGGTAACAGTCCTTG

Splice junction

GGGGTTTGCTAAATACTGGCAGGGCTTTCAGTATCCCCGTTACAG/GGCCGCTTCTGTTATAATAG  
GCACTGGCTGATCAGTGGCTGATTAATATGATGAAAAAGCCAAACCGTGGCCGCTTACGGCGGTGATT  
TCGGCGATACGCCAACGATGCCAGTTCTGTATGAAACGGTCTGGCTTTGCCGACCCACCCGATCCAG

2. NUCLEOTIDE SEQUENCES OF THE *trans*-SPLICED PRODUCT (195 bp)

BioLac-TR1

GGCTTCTACCTGGAGACGGCGCCGCTGATCCTTGGAAATACGGCCAGGGATGGTAACAGTCCTTG

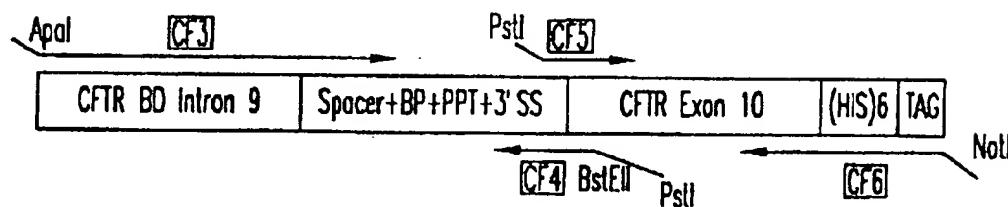
Splice junction

GGGGTTTGCTAAATACTGGCAGGGCTTACAG/TGGCTGCTGTTGCTGCTGCTGCTGCTGCTGCTGCT  
GAGGATGGGGGACATGGCATCCAGGAGCCACTTGGCCACGGTGGCG

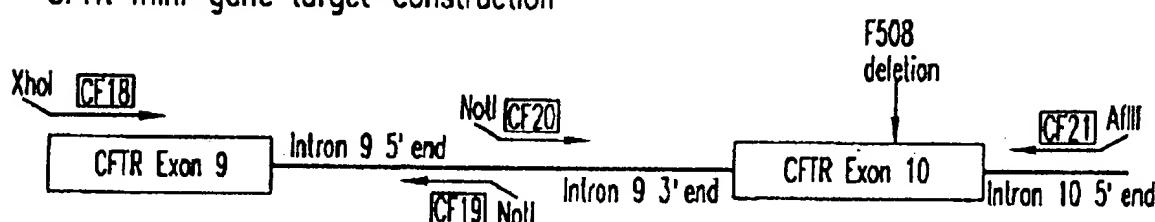
HCCR2

FIG. 12B

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

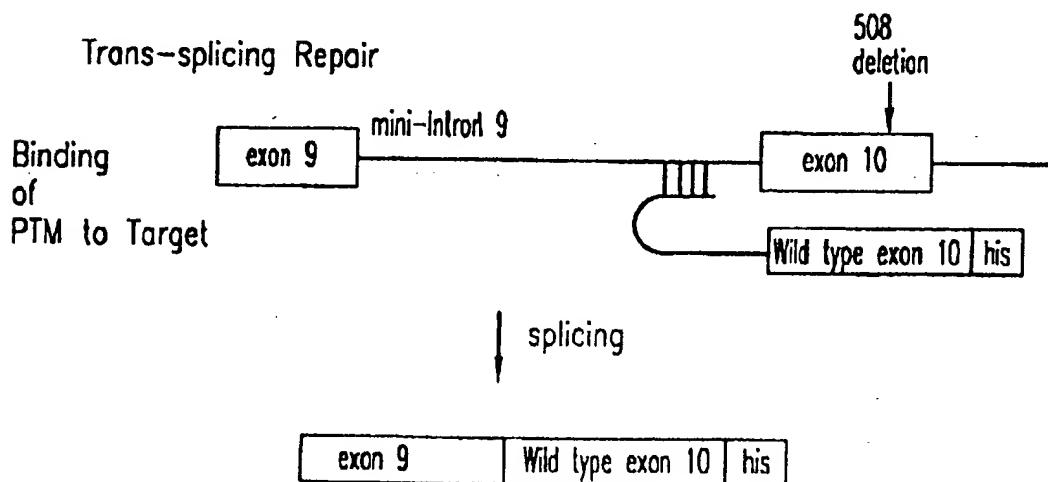


FIG.13

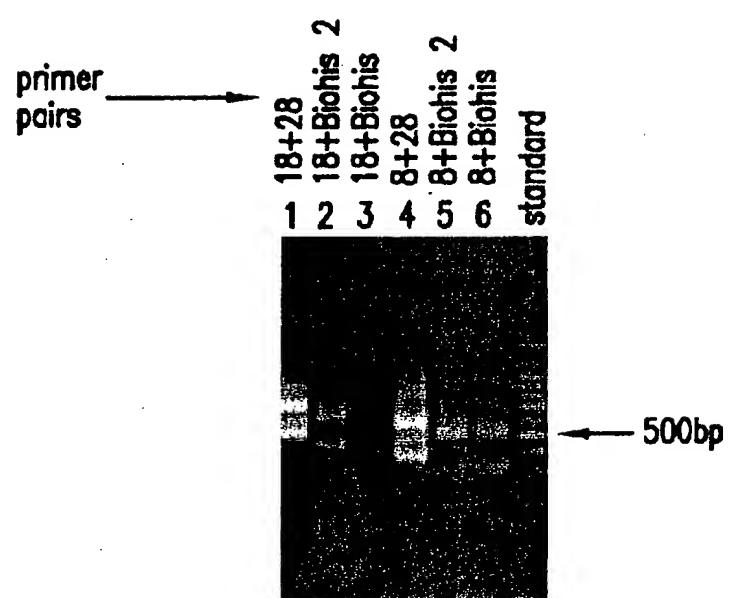


FIG.14

DNA sequence 500 b.p. GCTAGCGTTAA ... TGGCACTCCCCAC linear

## Positions of Restriction Endonucleases sites (unique sites underlined)

FIG. 15A

Restriction	Endonucleases	site	usage
Acc I	EcoR I	1	Nde I
Apa I	EcoR V	1	Nhe I
Apal I	Hae III	1	Not I
Avr II	Hae III	2	PfIM I
BamH I	Hinc II	1	Pst I
Ban II	Hind III	2	Pvu I
Bbe I	Hinf I	1	Pvu II

FIG. 158

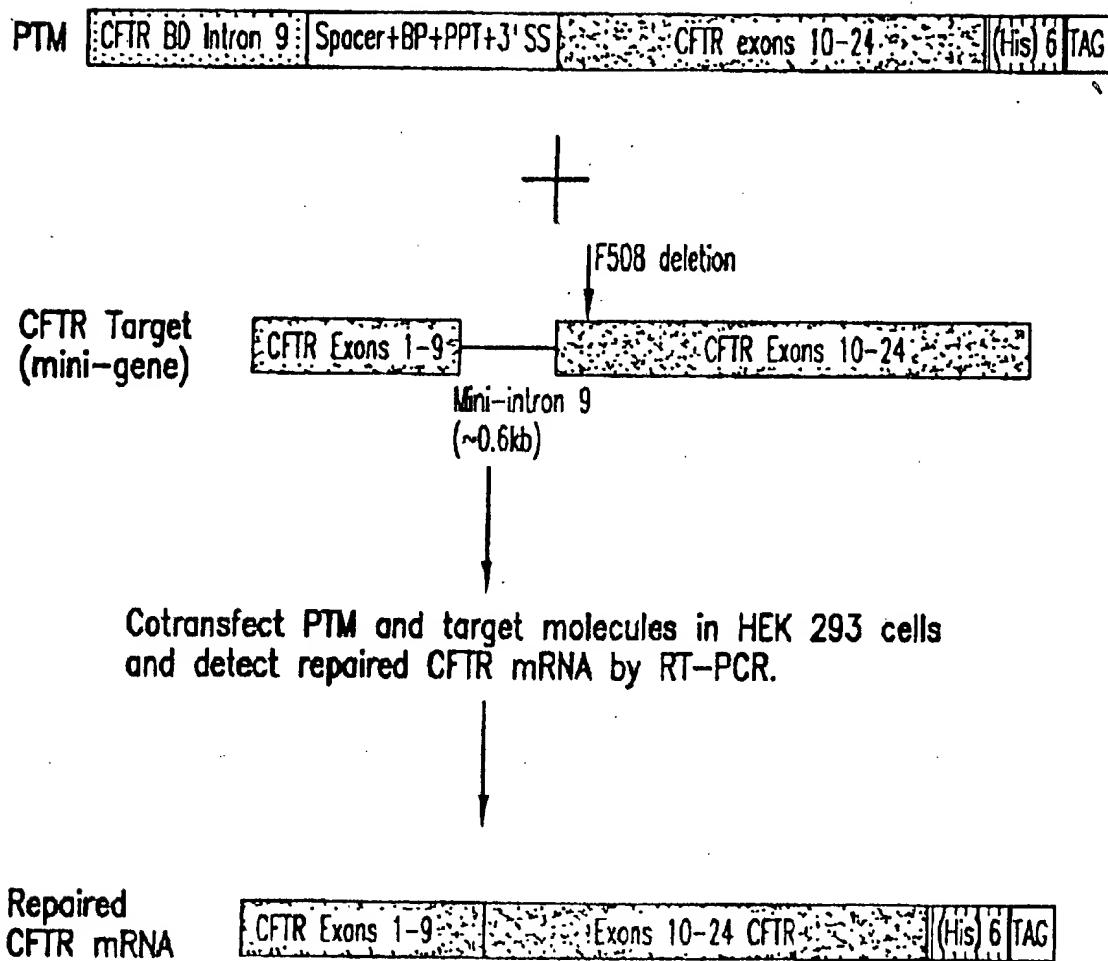


FIG. 16

Double Splicing  
PTM

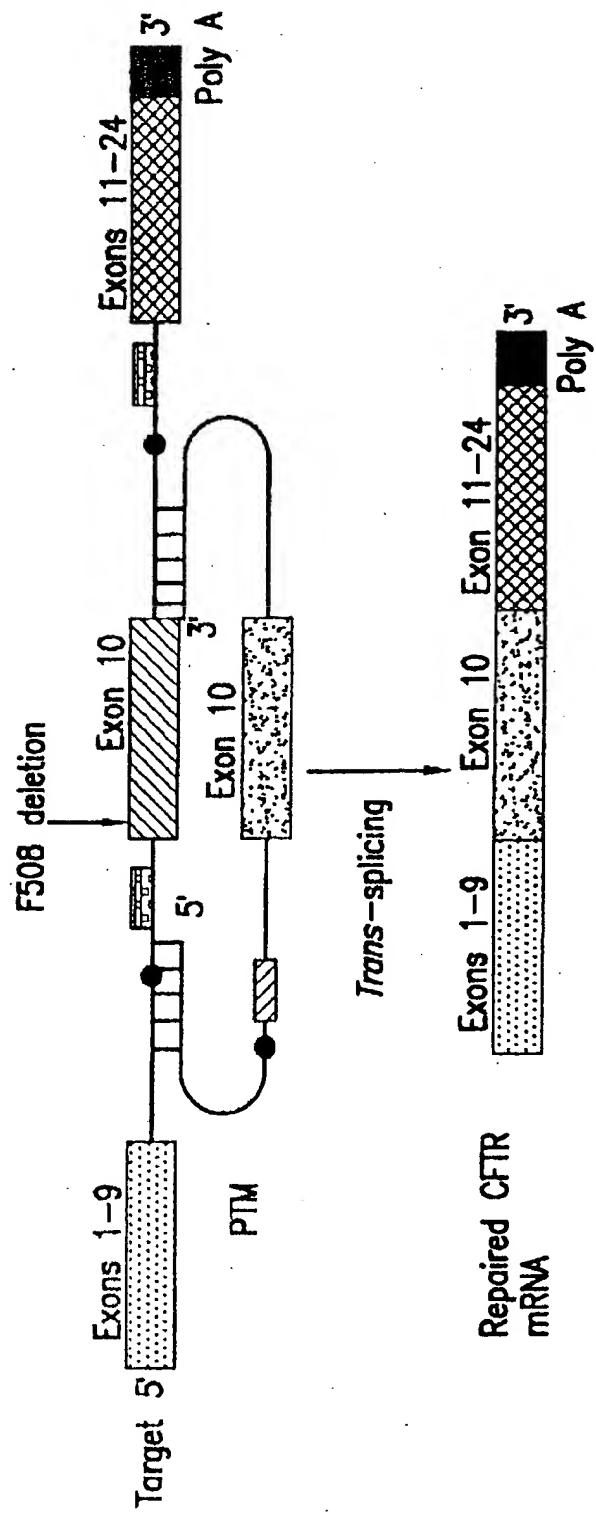


FIG. 17

DOUBLE TRANS-SPlicing SPECIFIC TARGET

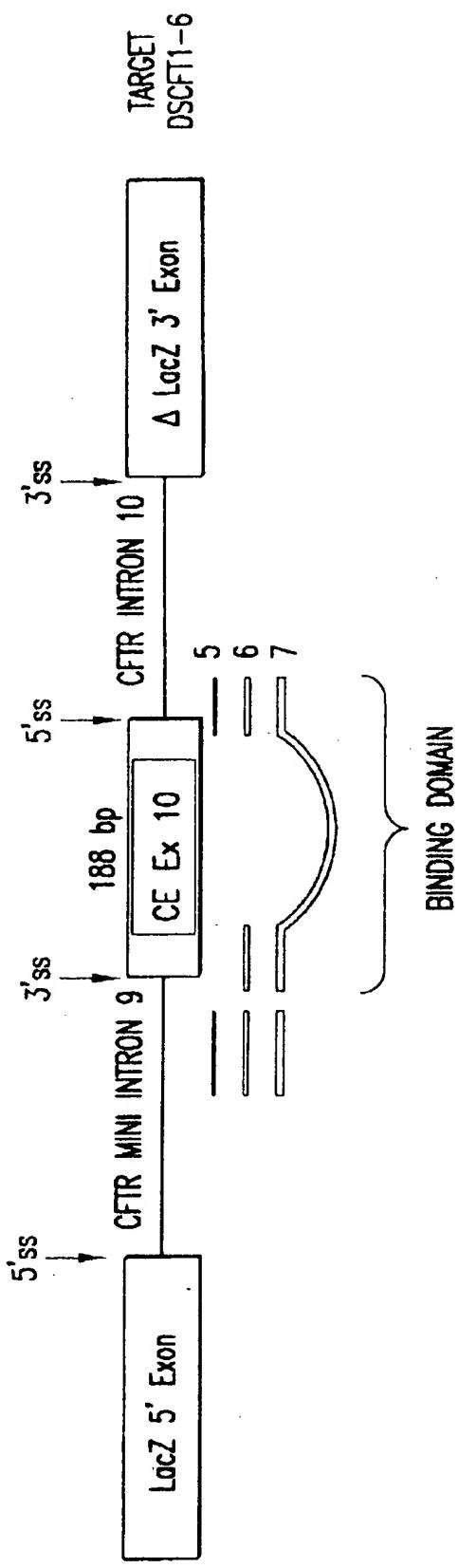


FIG. 18

## DOUBLE TRANS-SPlicing PTMs

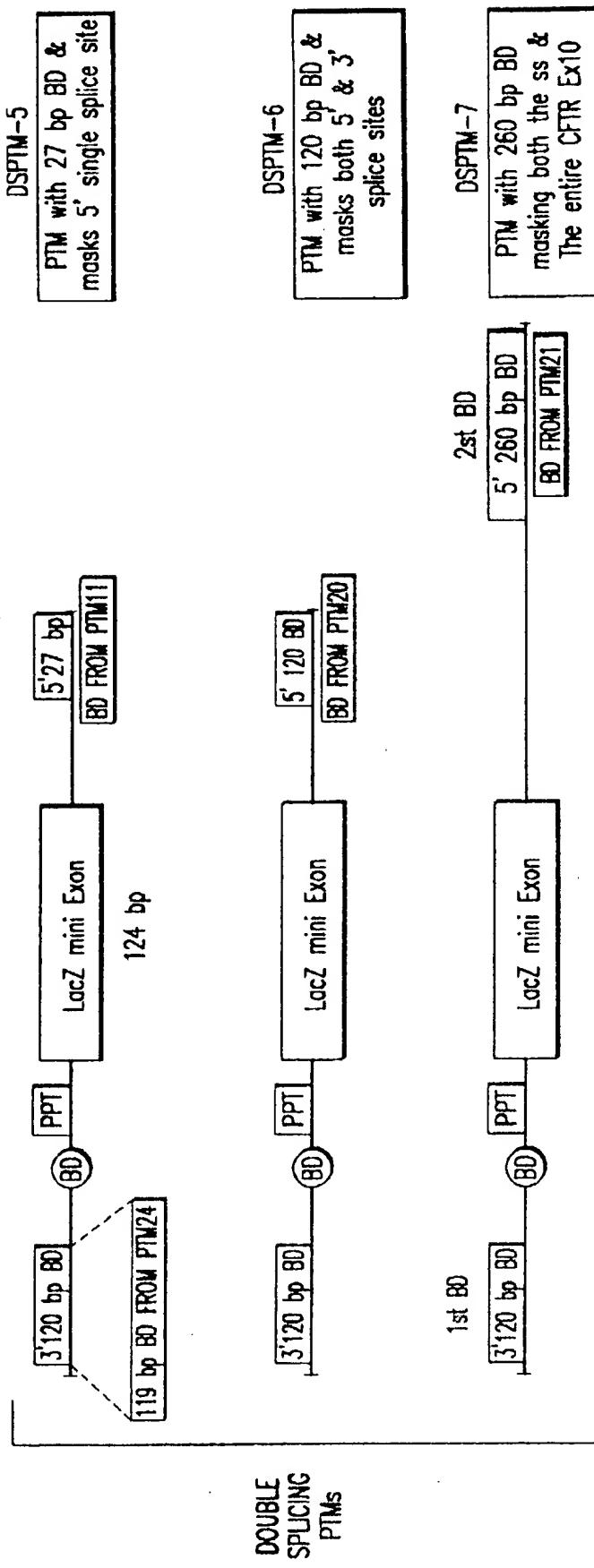


FIG. 19

DOUBLE TRANS-SPlicing  $\beta$ -GAL MODEL

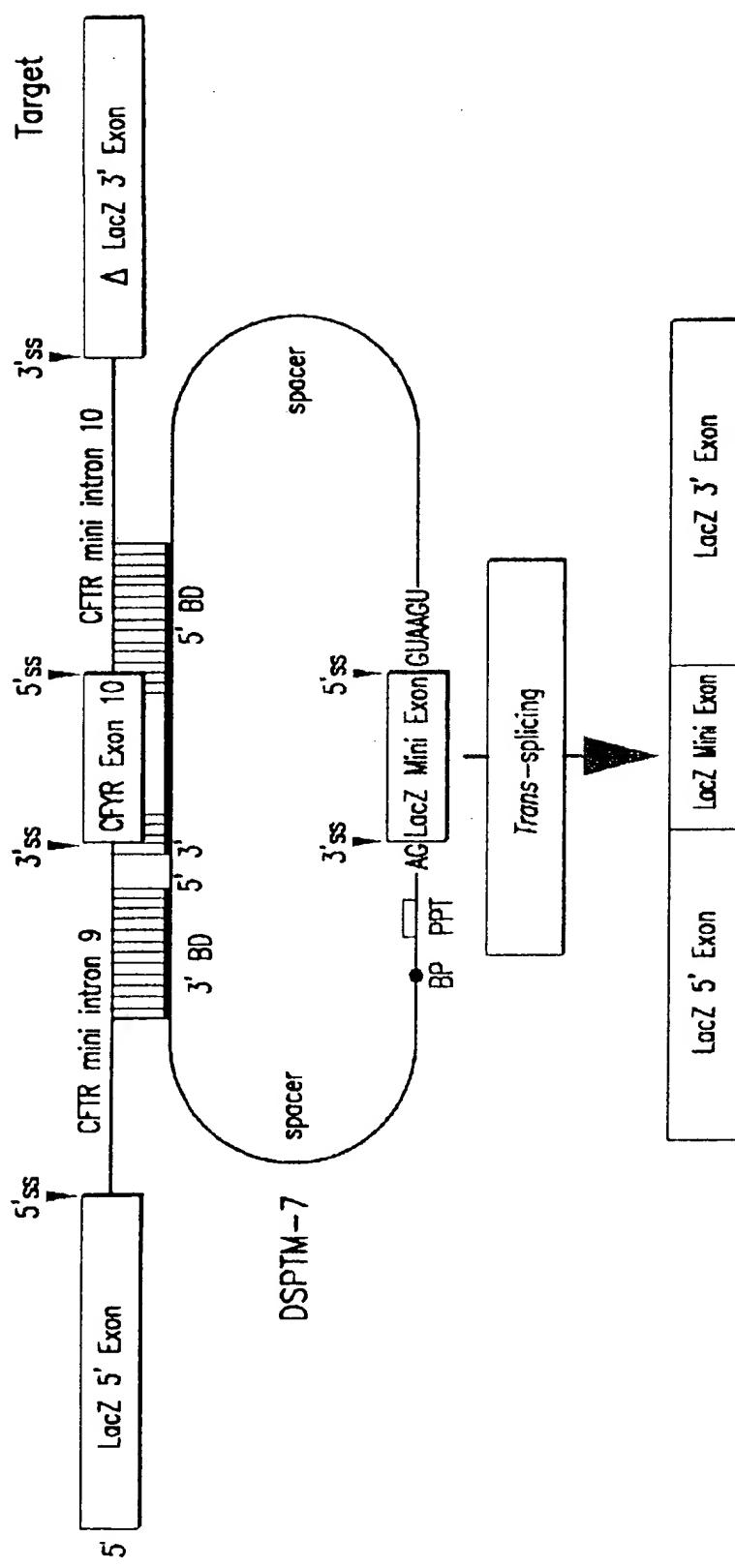
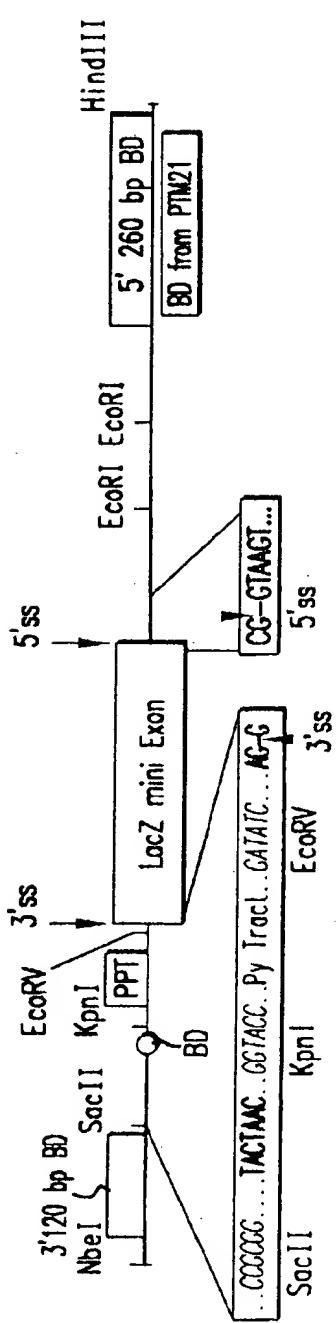


FIG. 20



(1) 3' BD (120 BP): CATTCACTTGCTCCAATTATCACTTAACCGAAAGTCATAATTCTTATTGTAAGATTCATTAACCTATTTGATT  
AAAATATTAAAATACTTCCCTGTTCACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATAAACCTTGCTCGAA

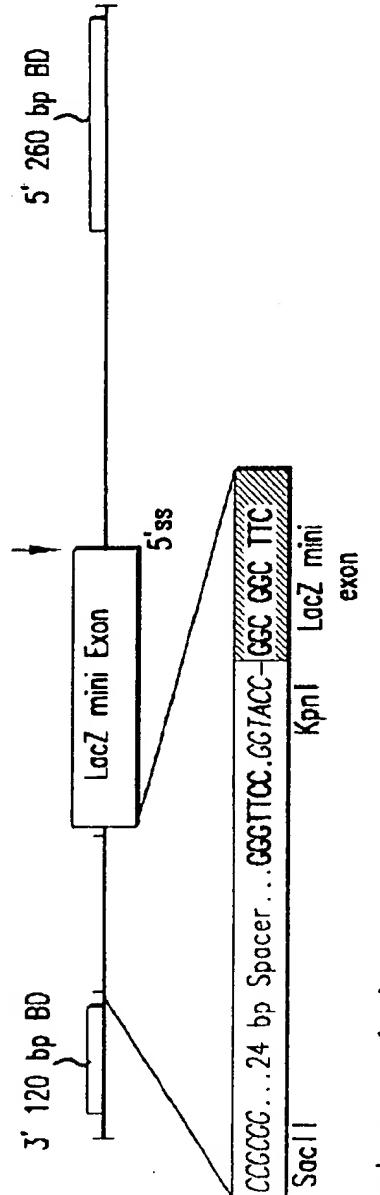
(3) Branch point, pyrimidine tract and acceptor splice site: TACTAAC 1 CGTACC TCTCTTTTTT GATACTCTGGCCTTCGATAGC  
3' ss LacZ mini exon

(4) 5' donor site and 2nd spacer sequence: TGA ACC GCTAAGT GTTATCACCGATAATGCTCTAACCTGATTCACGCTGCTTACGG  
CTAAGATCCACCGG

(5) 5' BD (260 BP): TCAAAAAGTTTCAAAATTCTTACCTCTTCAATTCTGCTTGTGATGACGCTTCGTTGATGAAATCTTCACTTGGAA  
ACACCAAATGAAATTCTTAAATGGTGGCTGGCAATAATCCCTGGAAACTGATAACACAATGAAATCTTCCACTGCTGCTAA  
AAAAACCCCTGAAATTCTCCATTTCCTCCATAATCACTCAATTACACTGAACCTGGAAAATAAAACCCATCAATTAACTCA  
TTATCAAATCACGC

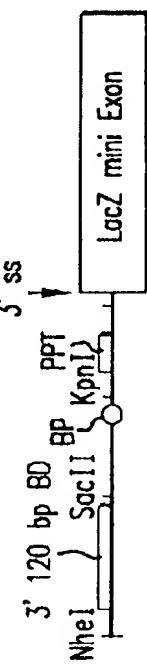
FIG. 21

DSPTM8: ( $\Delta$  3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



Mutants

PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)

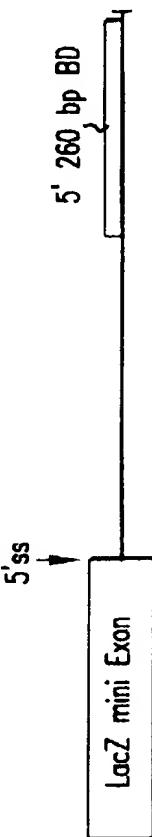


FIG.22

ACCURACY OF DOUBLE TRANS-SPlicing REACTION

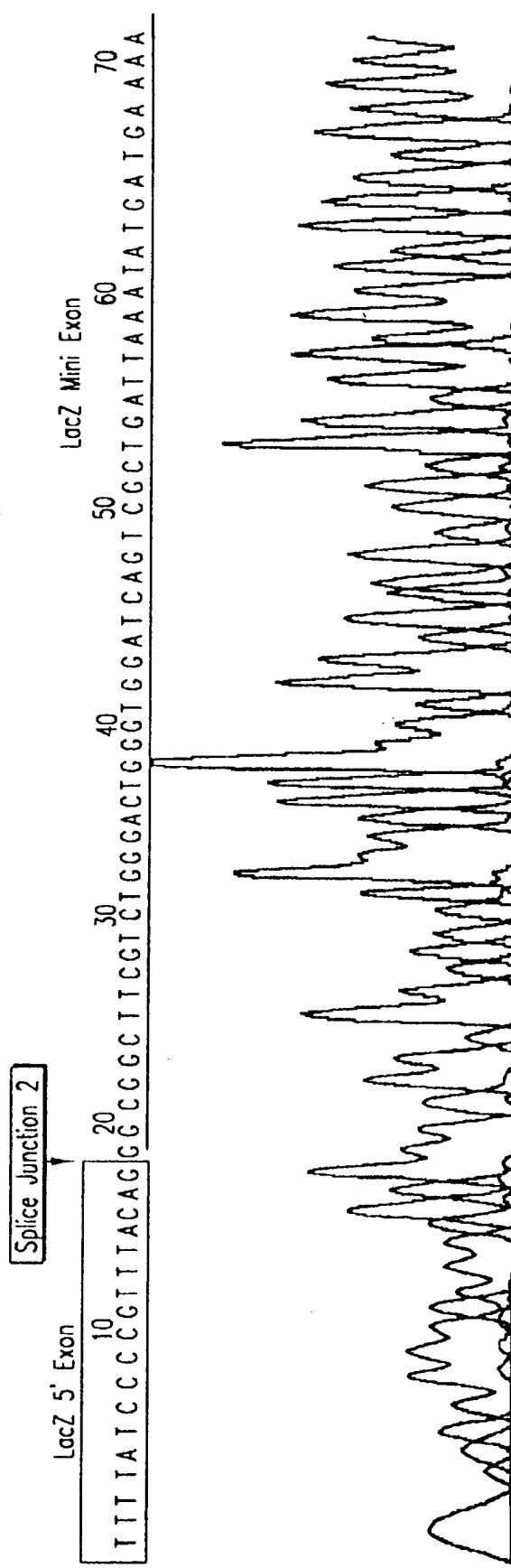


FIG.23A

## ACCURACY OF DOUBLE TRANS-SPlicing REACTION

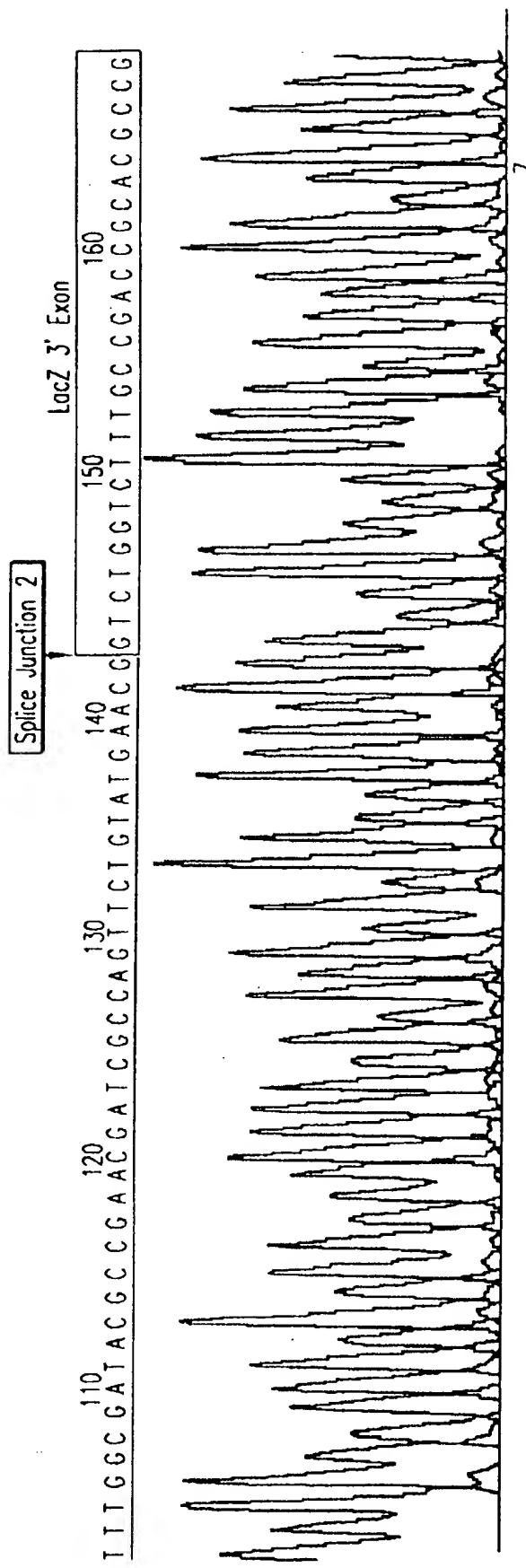
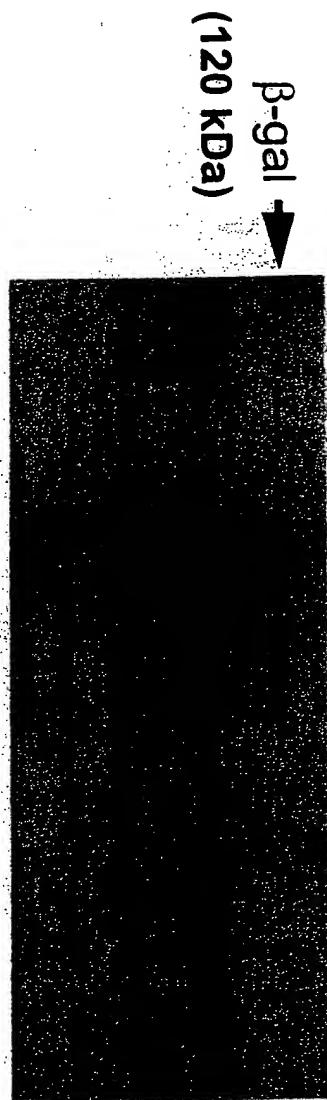


FIG. 23B

## Double Trans-splicing Produces Full-length Protein



- Lane 1: DSCFT1.6 Target alone 25 μg
- Lane 2: DSPTM7 25 μg
- Lane 3 Target + PTM #6 25 μg
- Lane 4: Target + PTM #9 25 μg
- Lane 5: Delta 3' splice mutant alone 25 μg
- Lane 6: Target + Delta 3' ss 25 μg
- Lane 7: Target+PTM29+30 (mutants) 25 μg

Figure 24

RESTORATION OF  $\beta$ -GAL FUNCTION BY DOUBLE TRANS-SPlicing

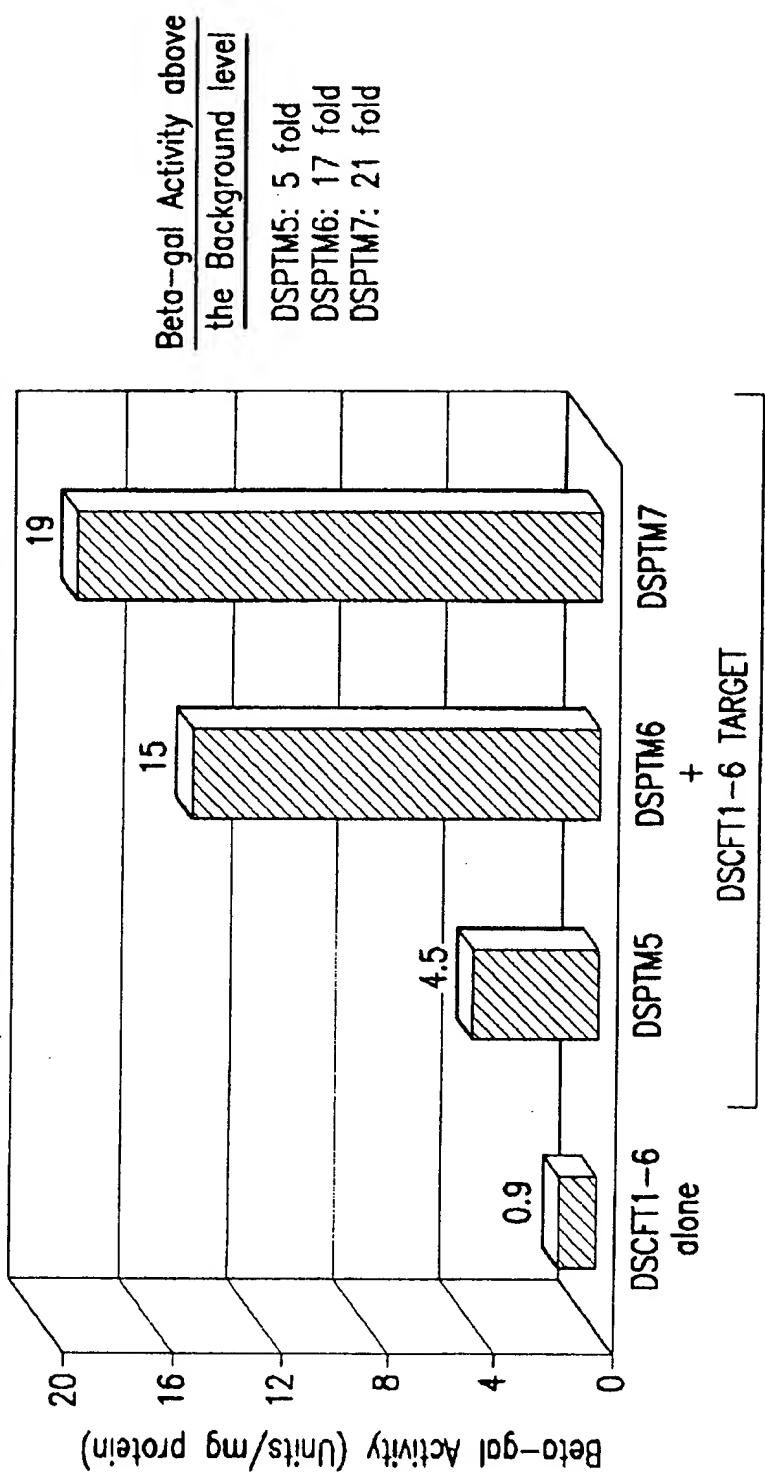


FIG. 25

RESTORATION OF  $\beta$ -GAL ACTIVITY IS DUE TO DOUBLE RNA  
TRANS-SPLICING EVENTS

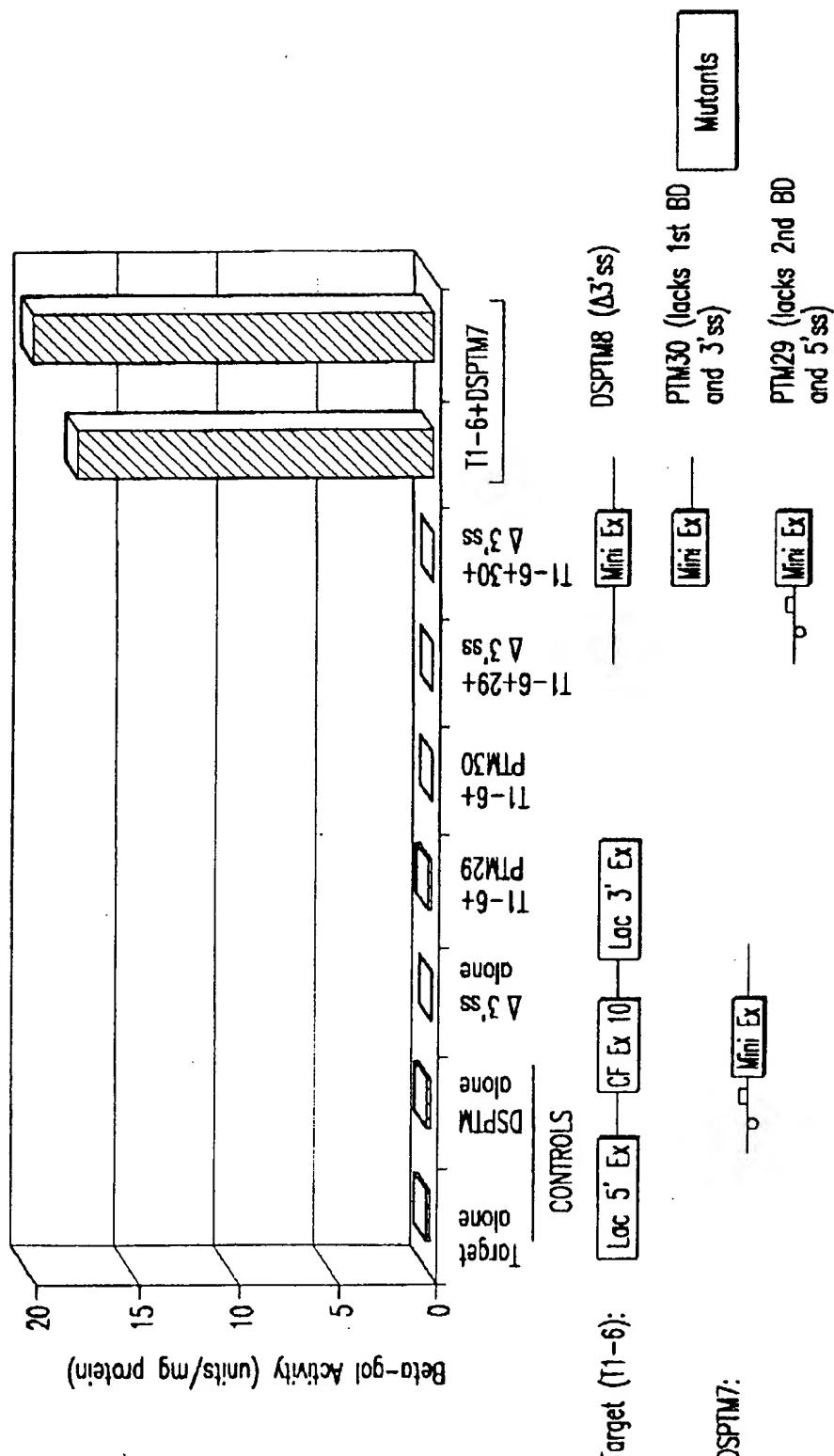


FIG. 26

## DOUBLE TRANS-SPlicing: TITRATION OF TARGET &amp; PTM

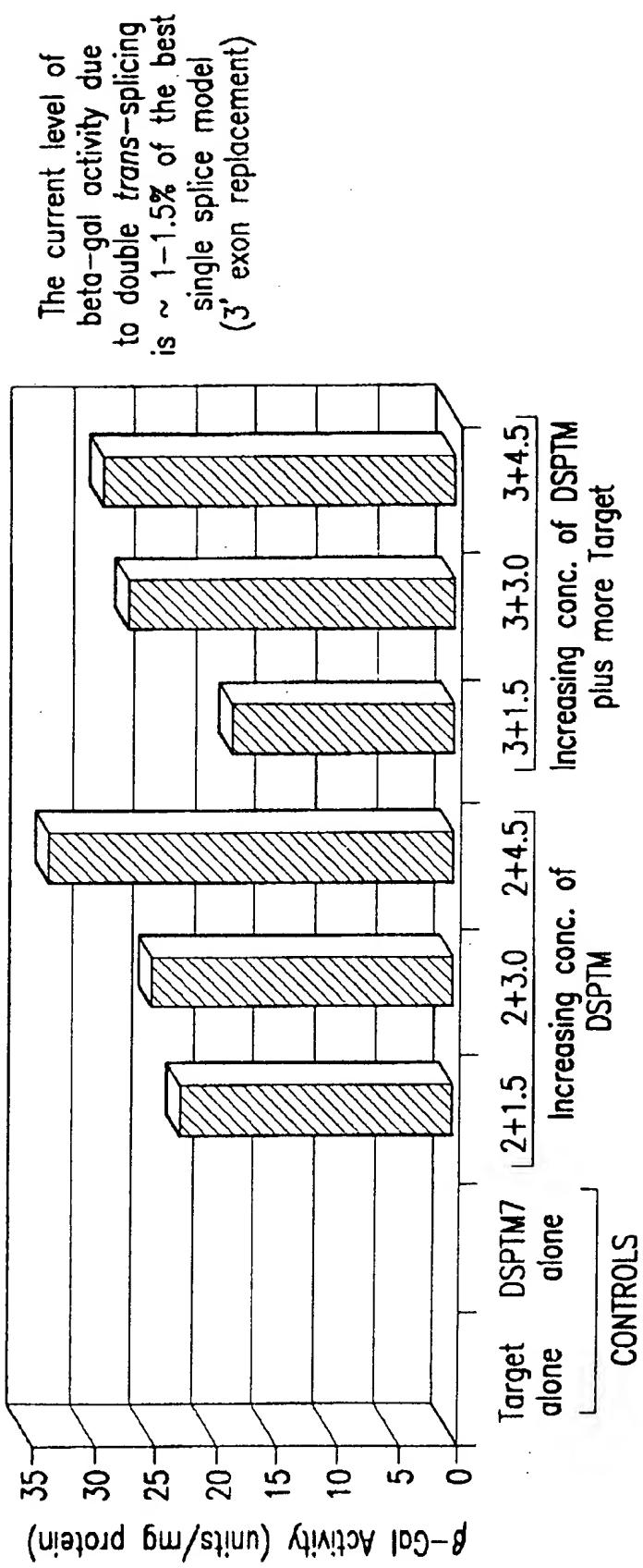


FIG. 27

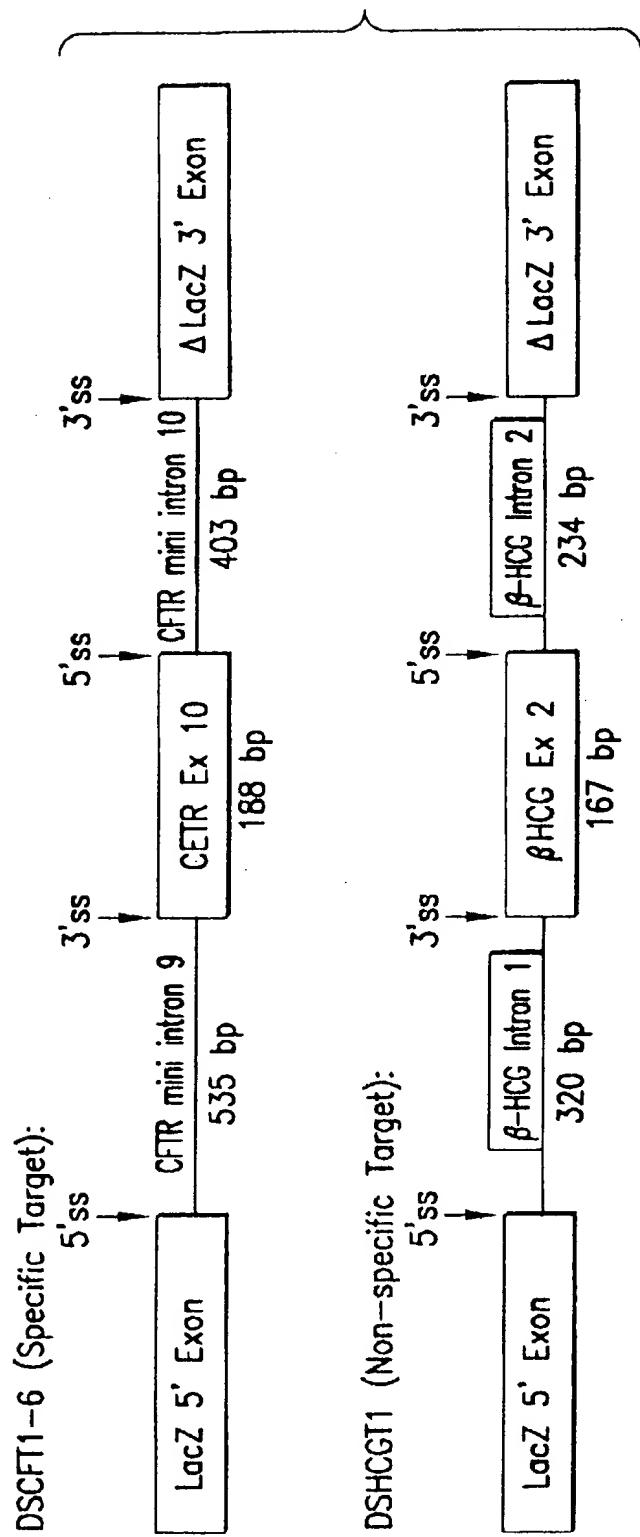


FIG. 28

## SPECIFICITY OF DOUBLE TRANS-SPlicing REACTION

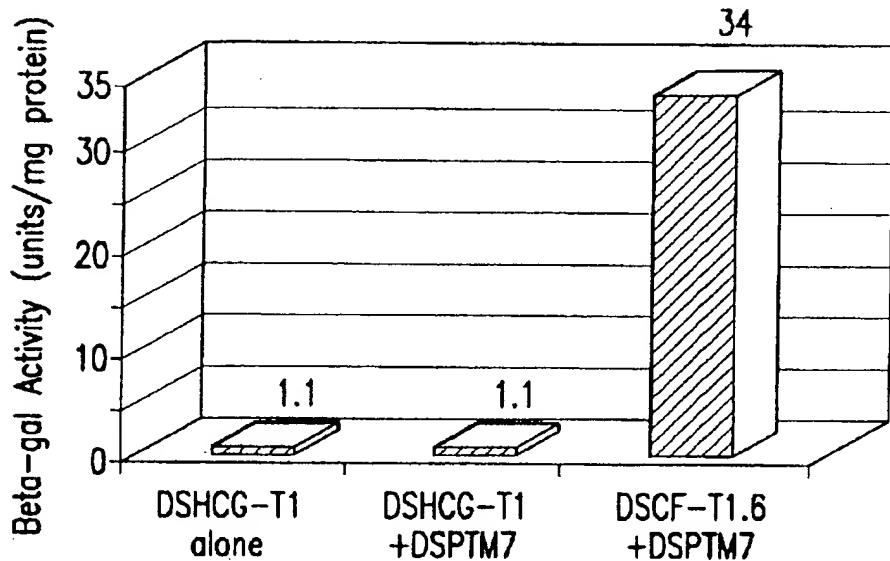


FIG.29

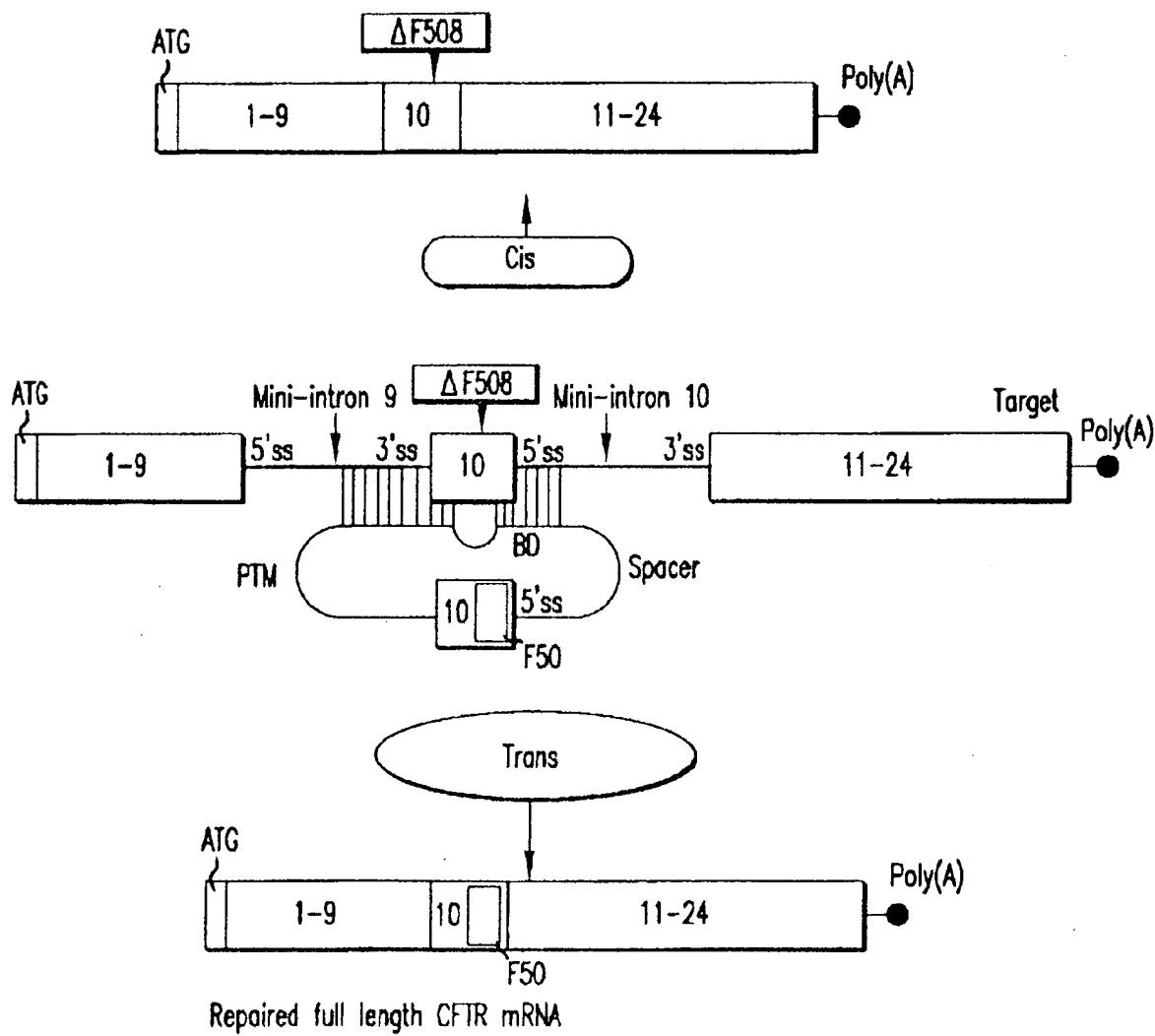
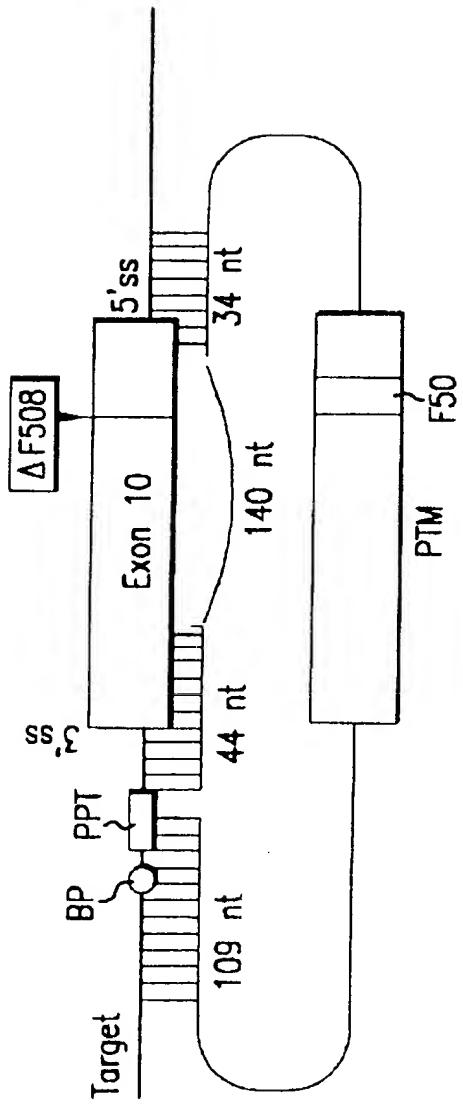


FIG.30

PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target

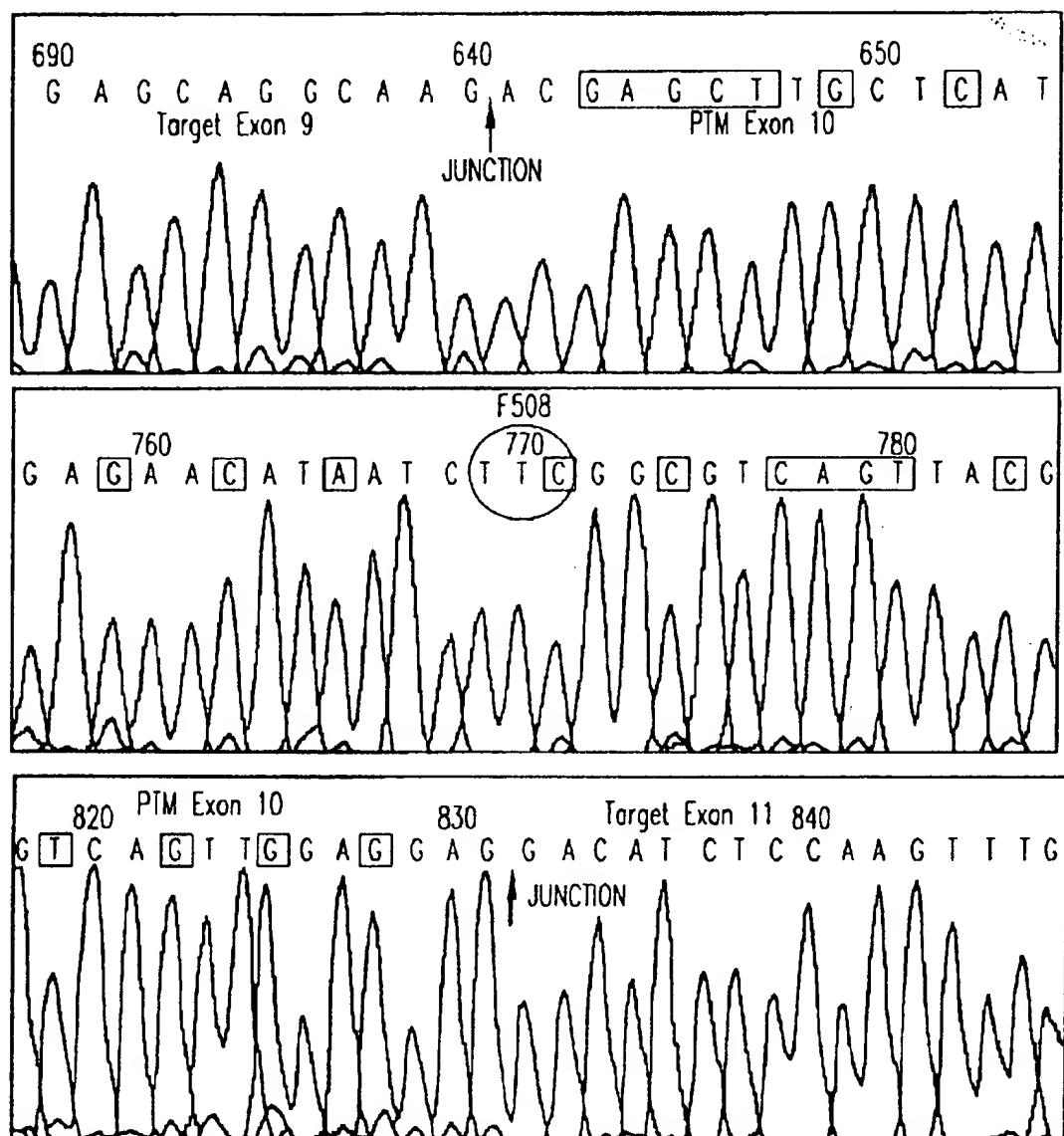


ACGAGCTTGGCTCATGATGATCATGGCGAGITAGAACCAAGTGAAGGCCAAGATCAAACATCCG  
GGCGCAT**TAGCTT**GGC**CAA**TT**CAGT**GGAT**CA**TGGCCGGTACCAT**CA**AGGAGAACAT**AA**T  
C7TGGCC**TAGTT**AGG**AA**G**AT**CC**CT**AT**CC**CT**AA**CC**CT**AT**CC**CT**AA**CC**CT**GG**GT**ATTAGGCC**CT**GG**GT**ICAGT**GG**AGG**AC**

MCU in exon 10 of PTM  
88 OF 192 (46%) bases in PTM exon 10 are not complementary to  
its binding domain (bold and underlined).

FIG. 31

Sequence of a double  
Trans-spliced product



□ = MCU in  
PTM exon 10

FIG.32

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

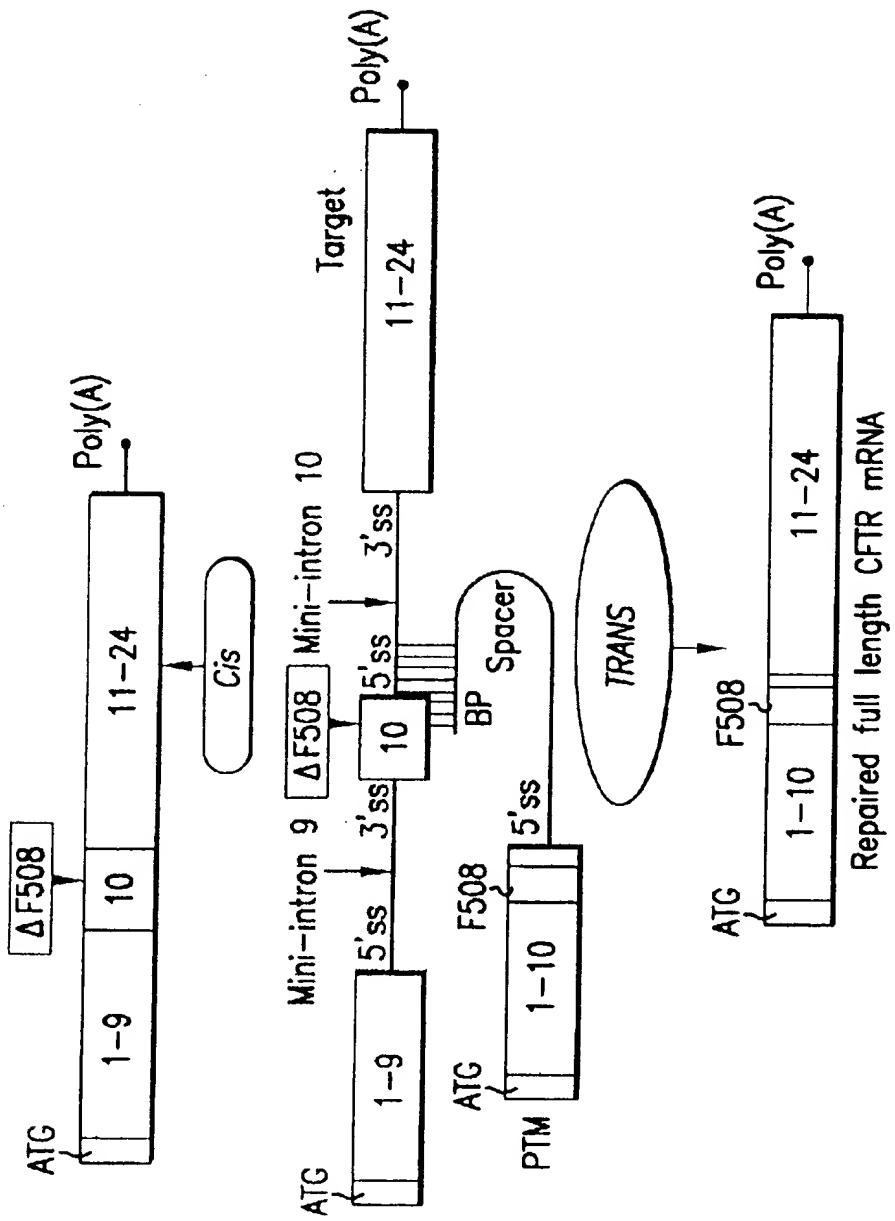


FIG. 33

PTM with a short binding domain masking a single splice site in a mini-gene target.

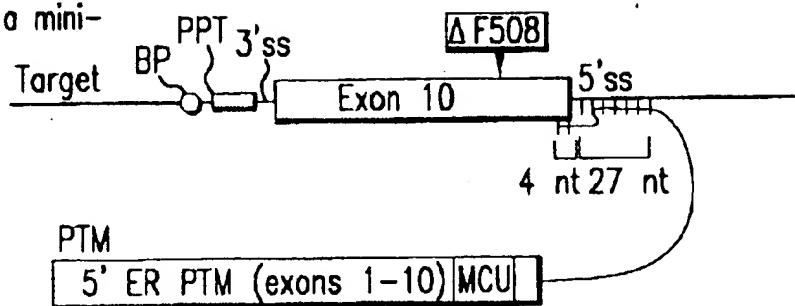


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

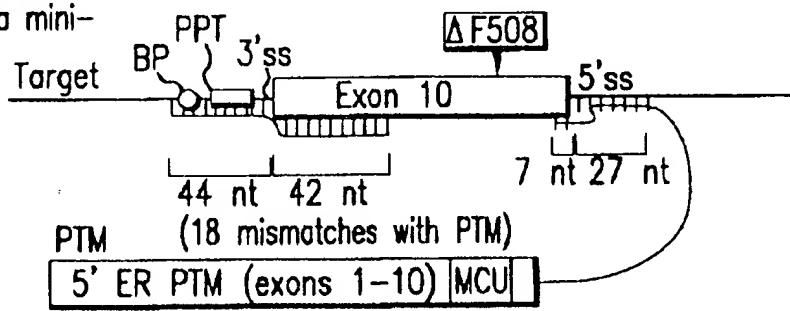


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

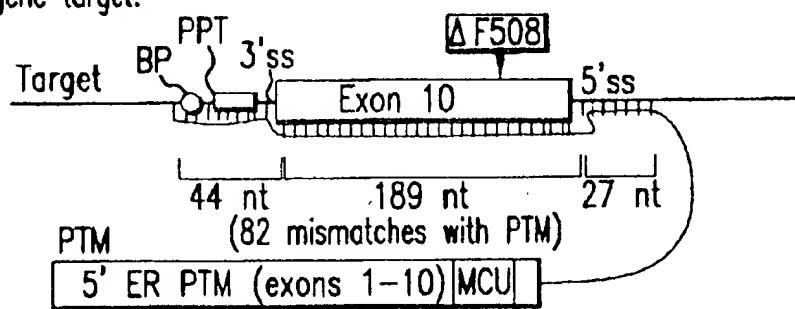
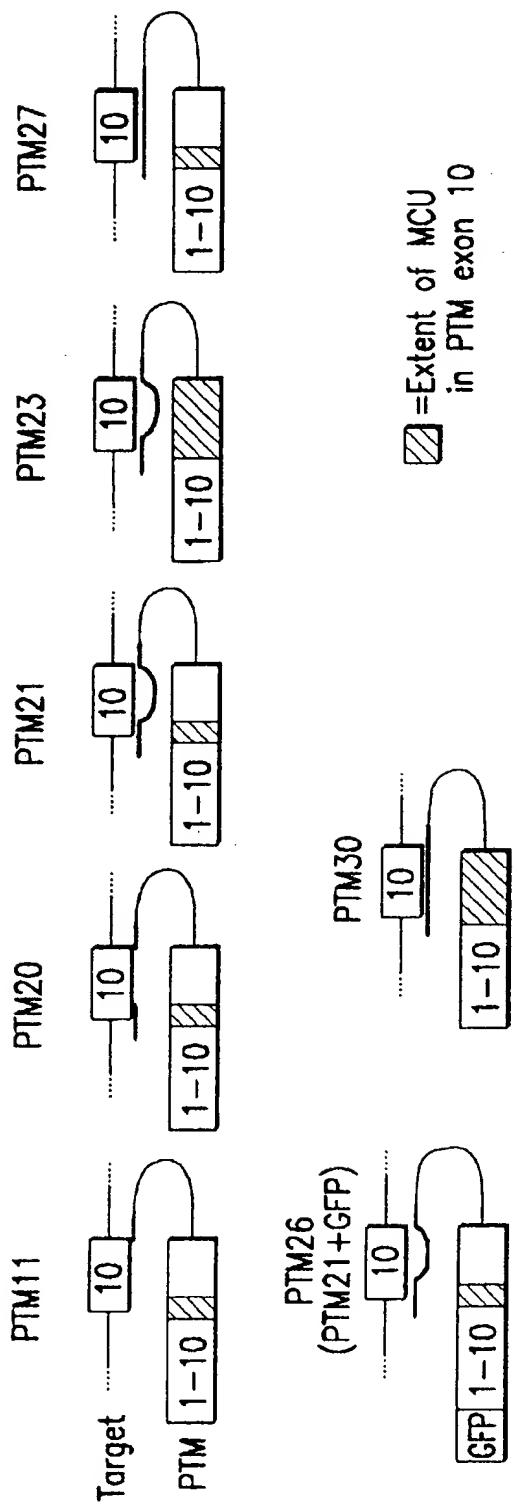


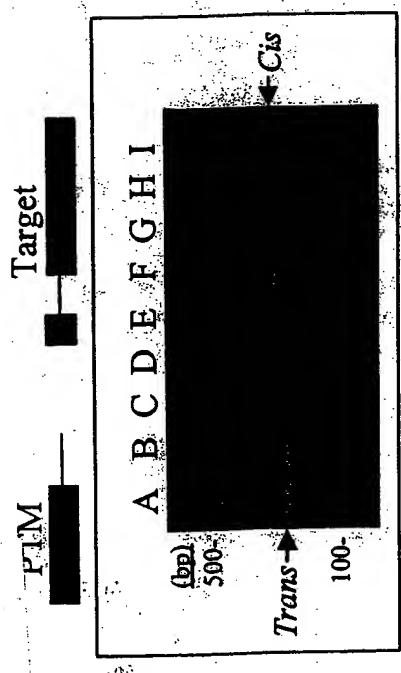
FIG.34C



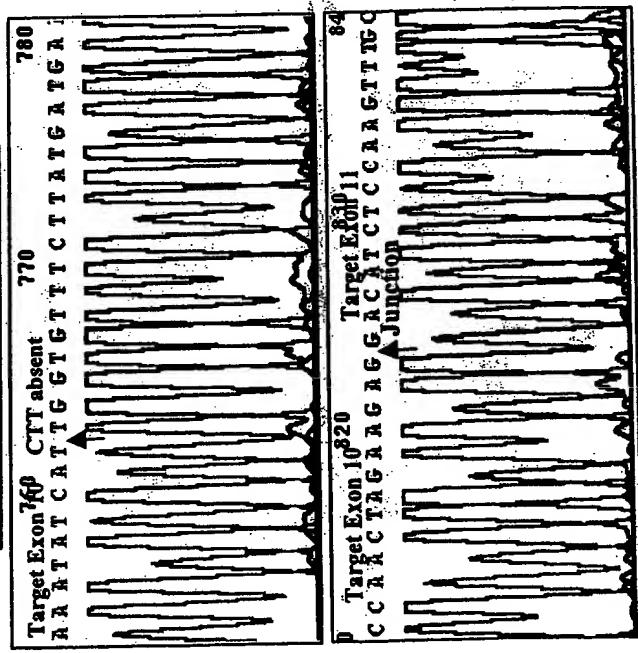
MCU in exon 10 of PTM  
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCTCATGATGATGATGAGTAAACCAAGTGAAGGCAAGATCAAACATTCGG  
GGGGCAATCAGCTTITGCAGGCCATTTCAGTTCCATCATGCCGCTACATCAAGGAGAACATAAT  
CTTGGCCGTCAGTTACGGAGTACGGAGTACGGCTAICGCTGGTGATTAAAGCCCTGICAGTTGAGGAG

FIG. 35

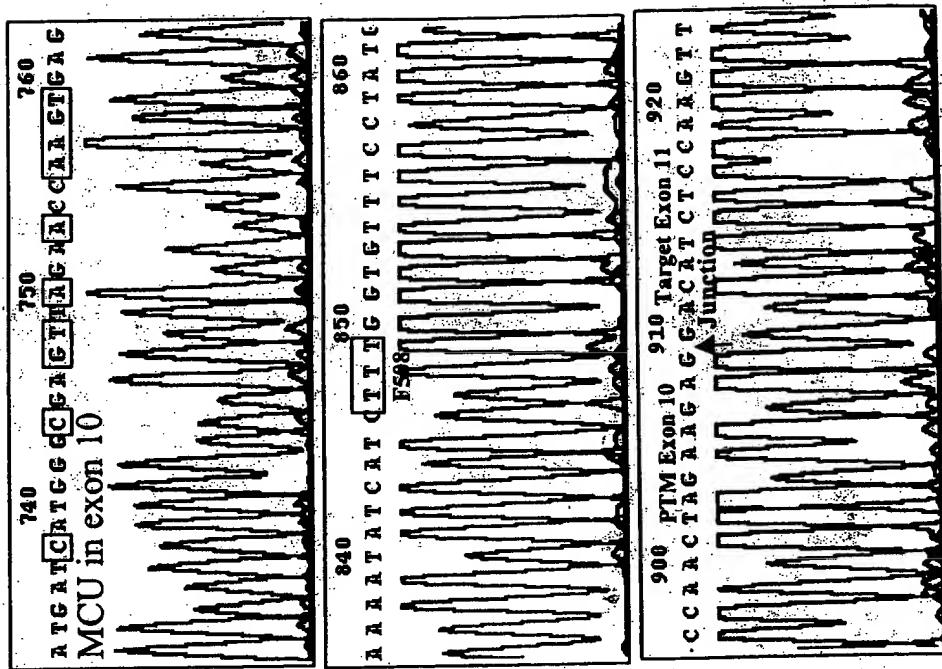


**Cis-spliced product**  
[Primers CF1 + CF111]



93 2nd 14

**Trans-spliced product**  
[Primers CF93 + CF111]



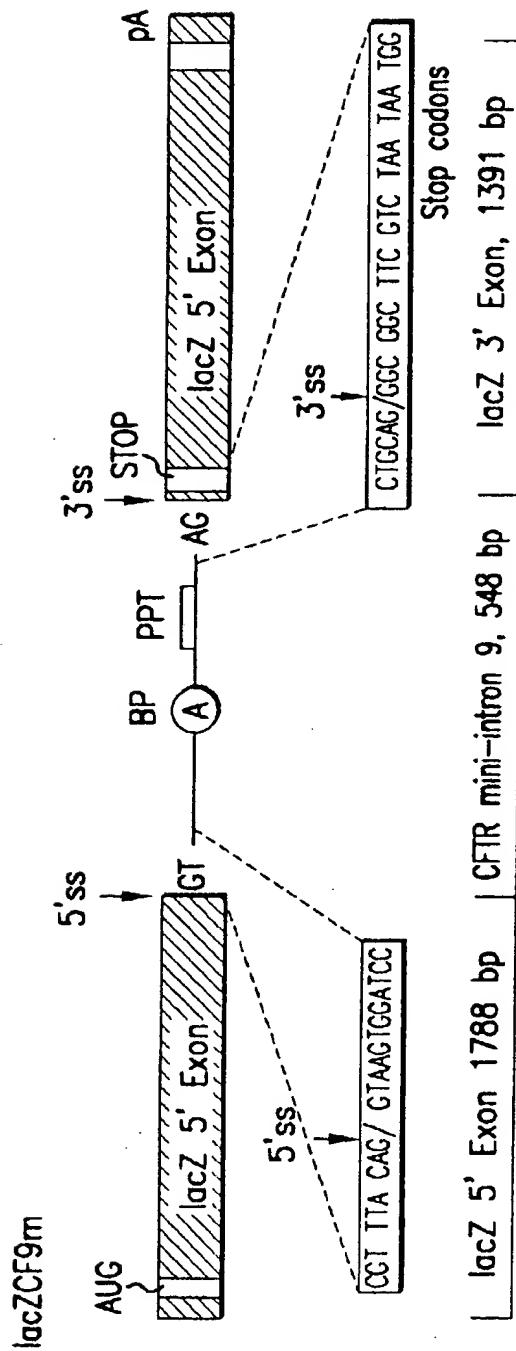
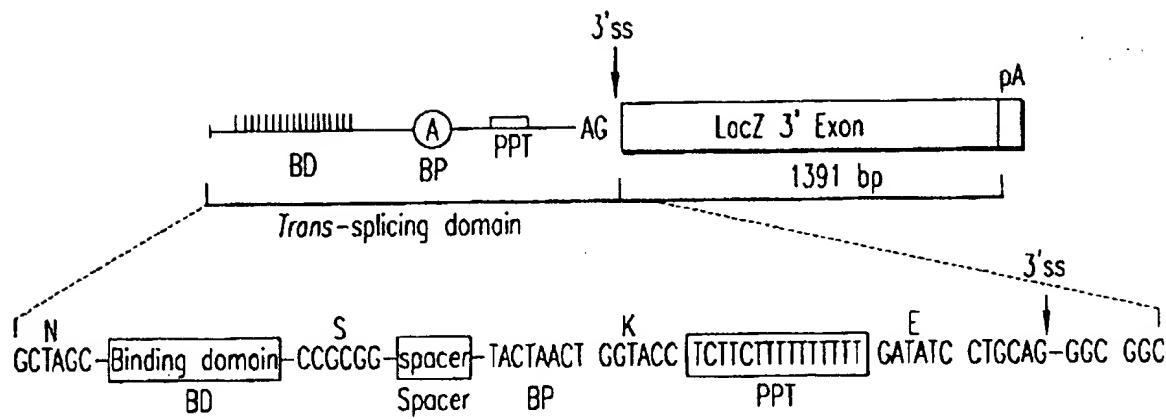


FIG. 37A



PCT/US2000/016606

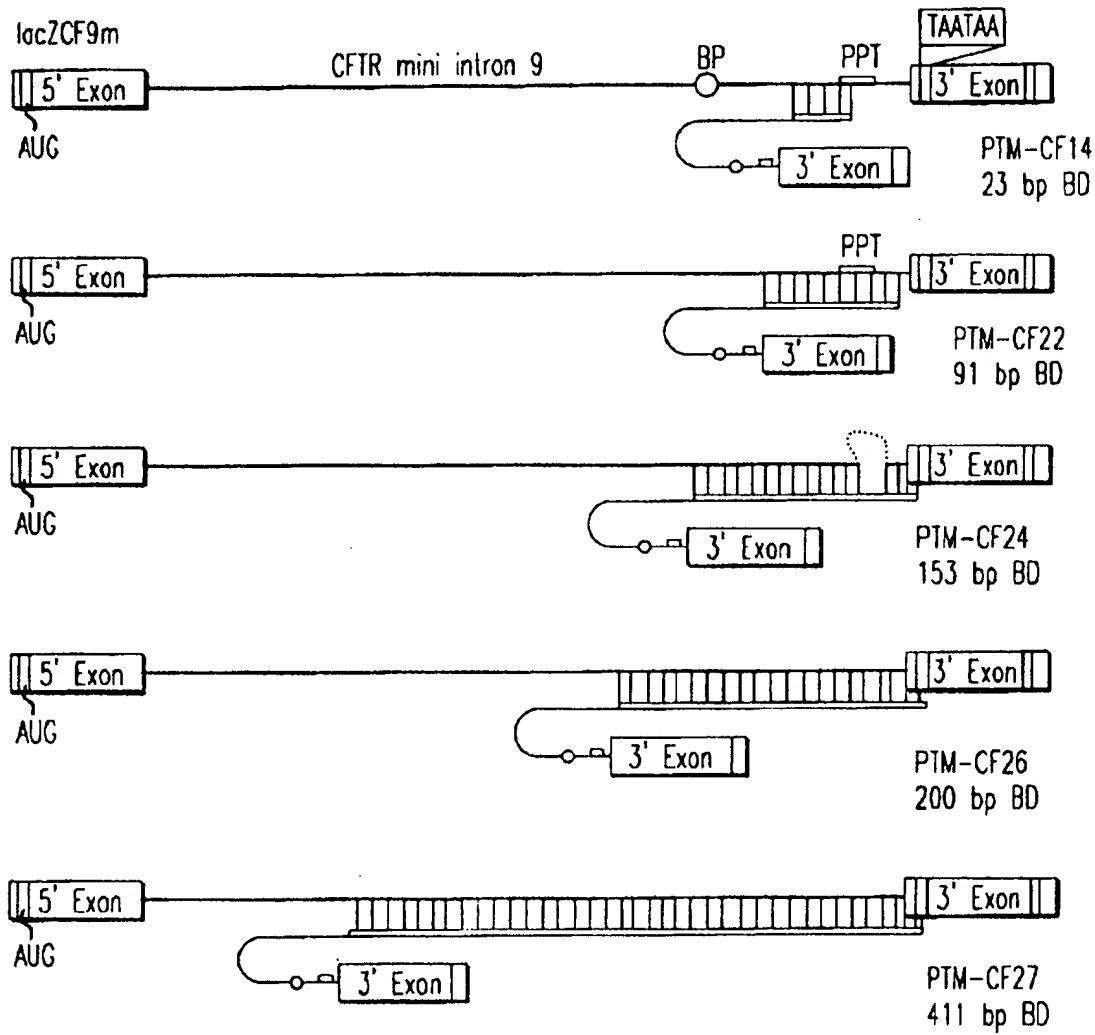


FIG.37B

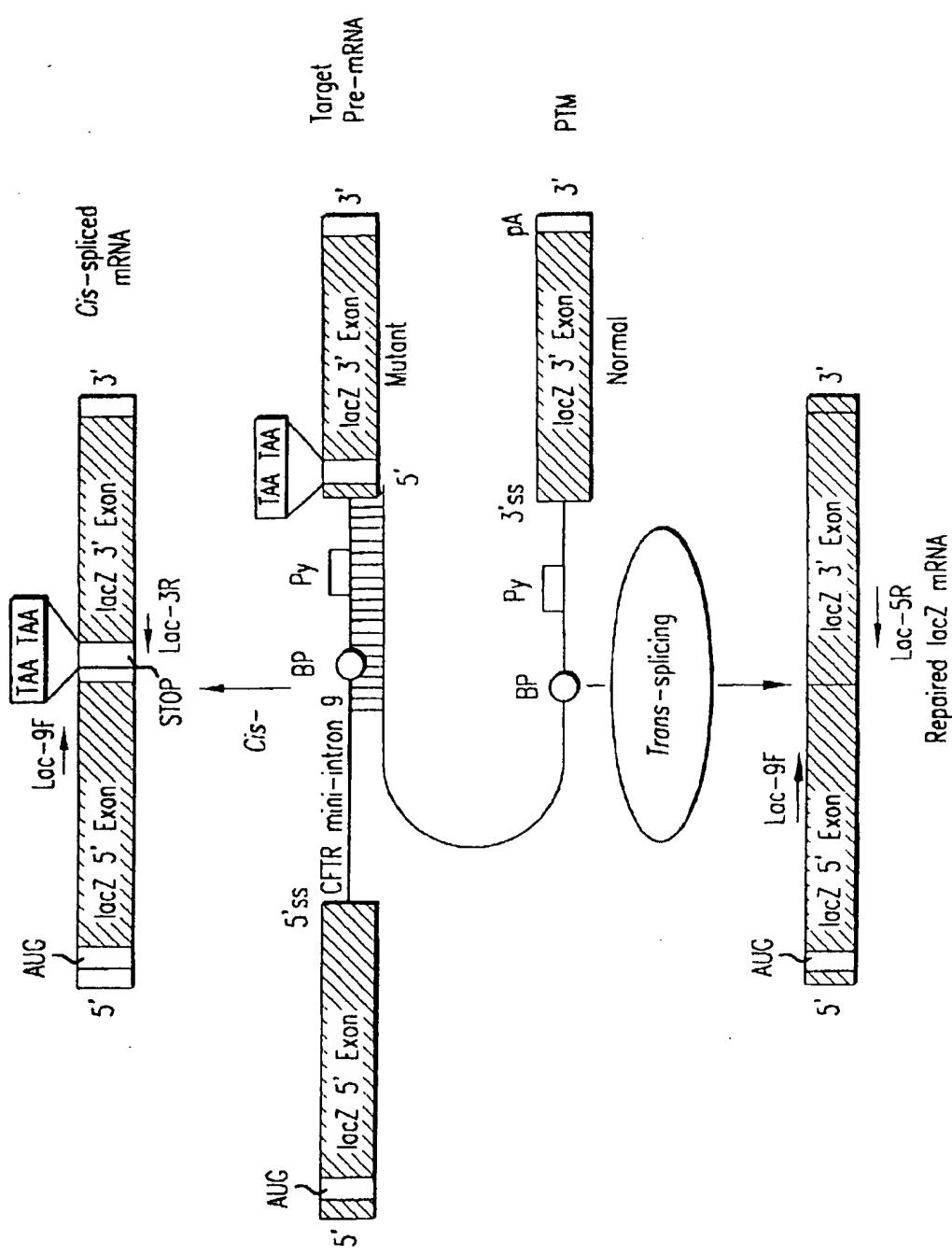
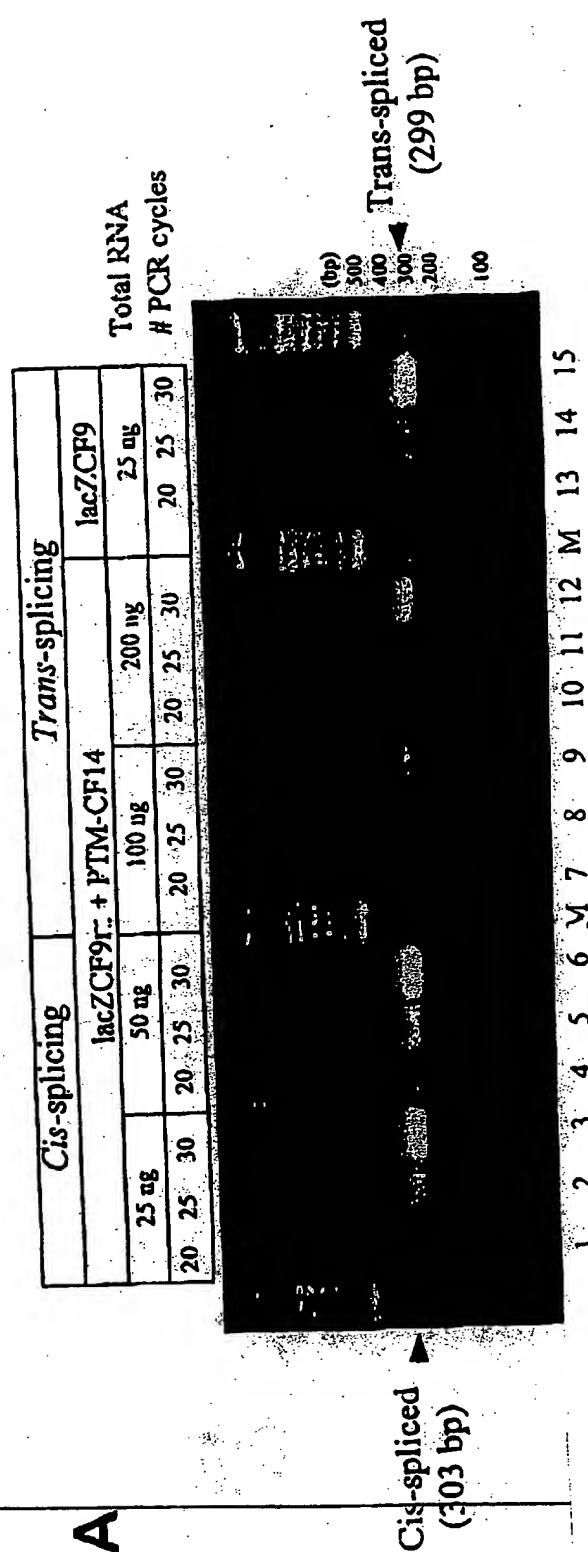
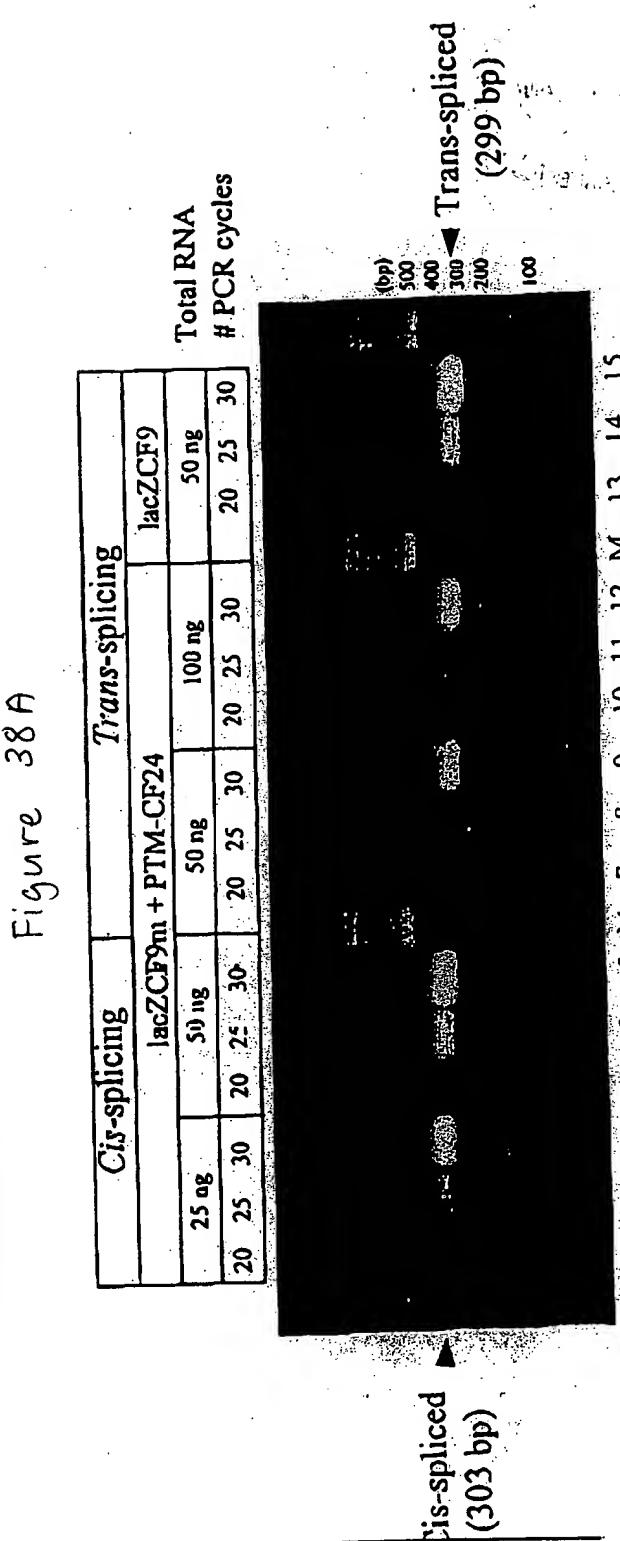


FIG. 37C



4



**B**

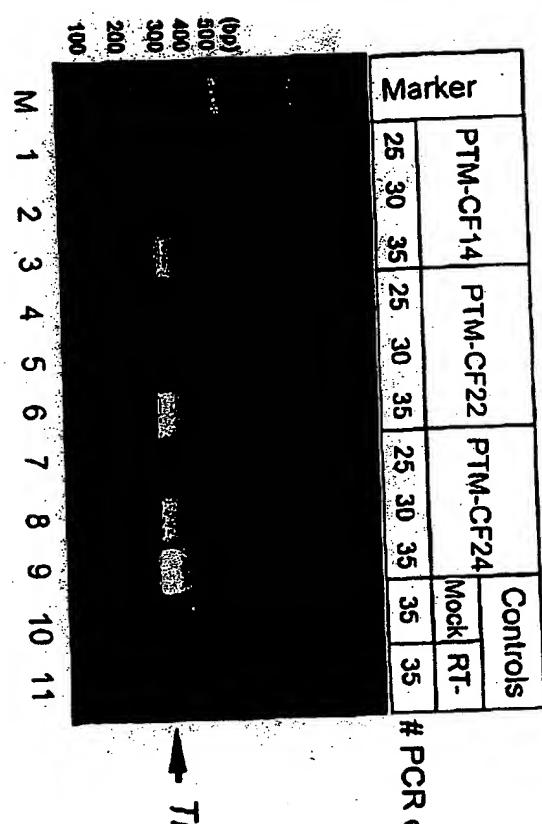


Figure 38B

卷之三

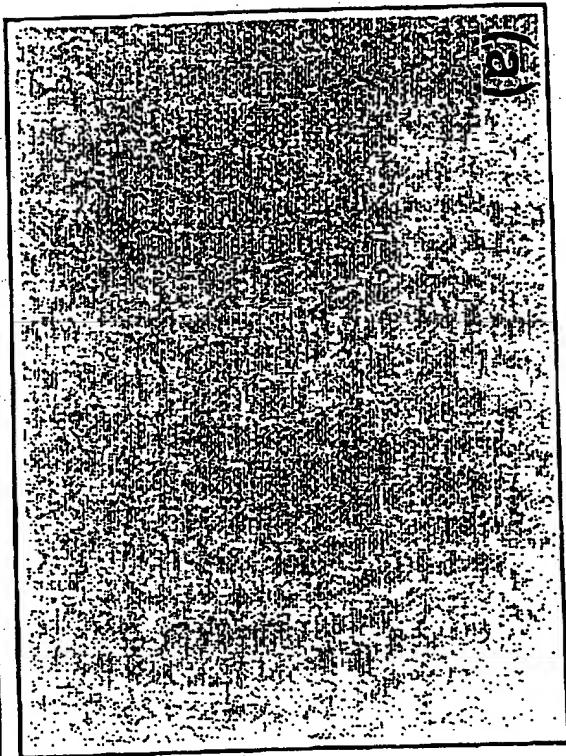
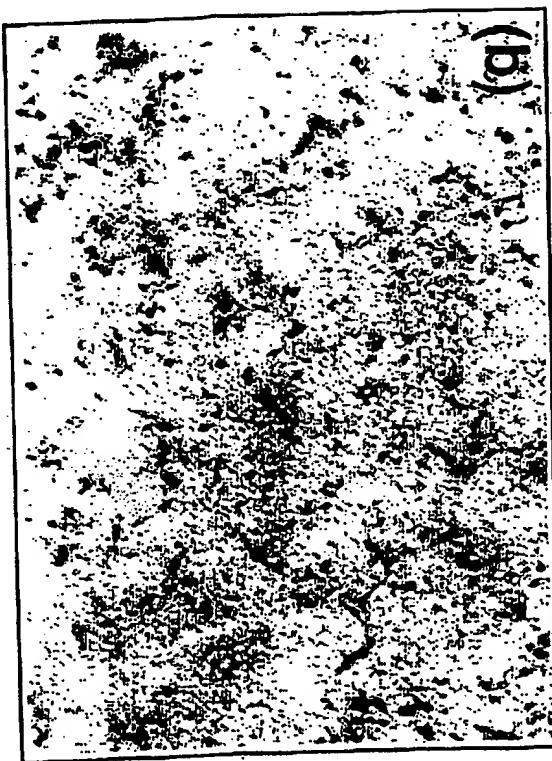


Figure 40A

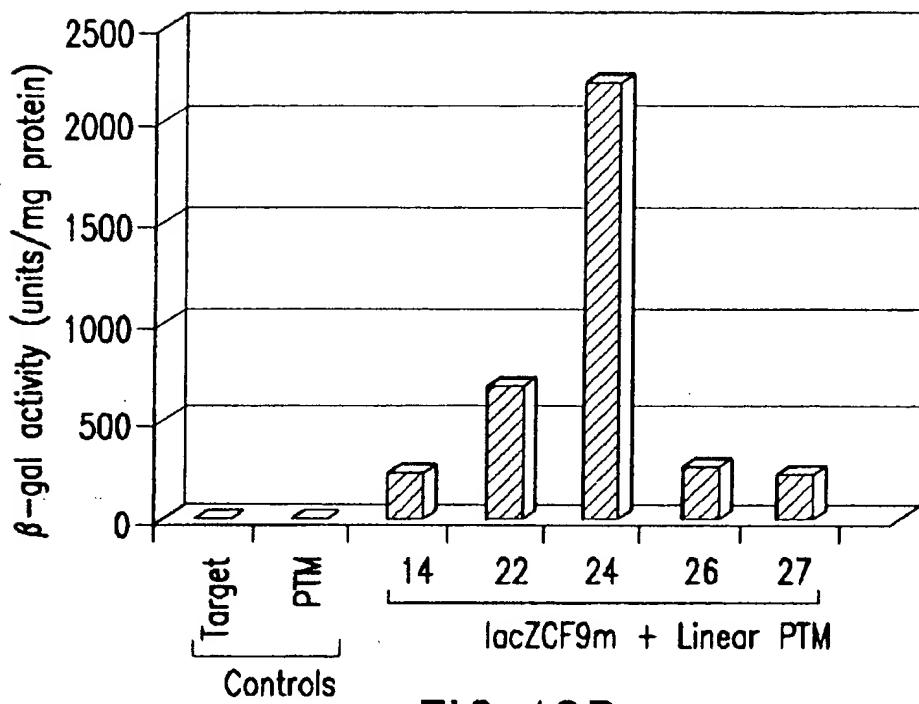


FIG.40B

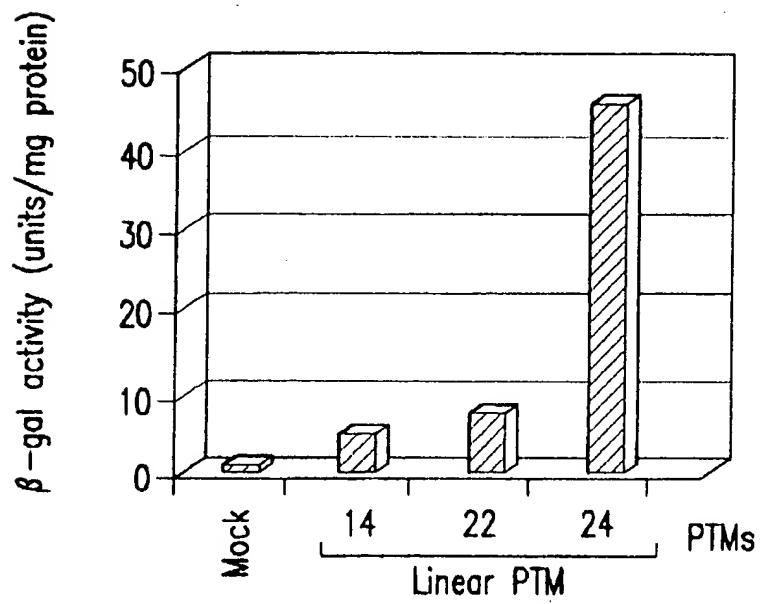


FIG.40C

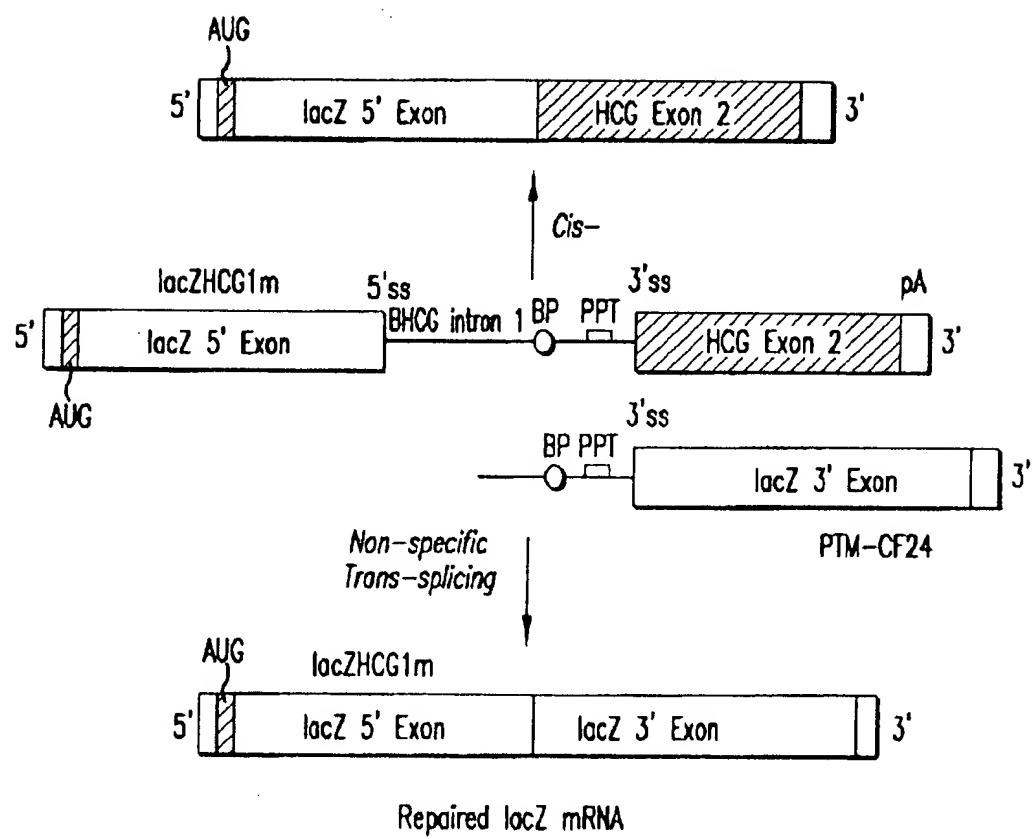


FIG.41A

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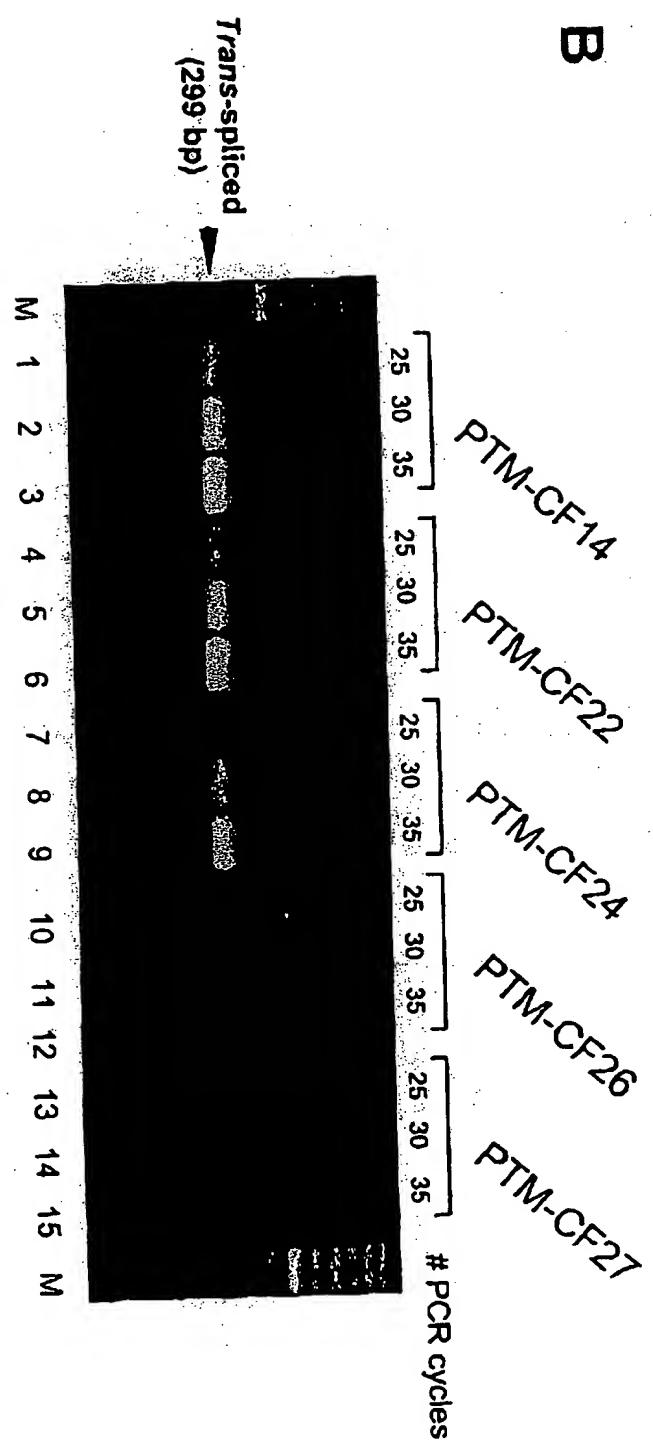


Figure 4KB

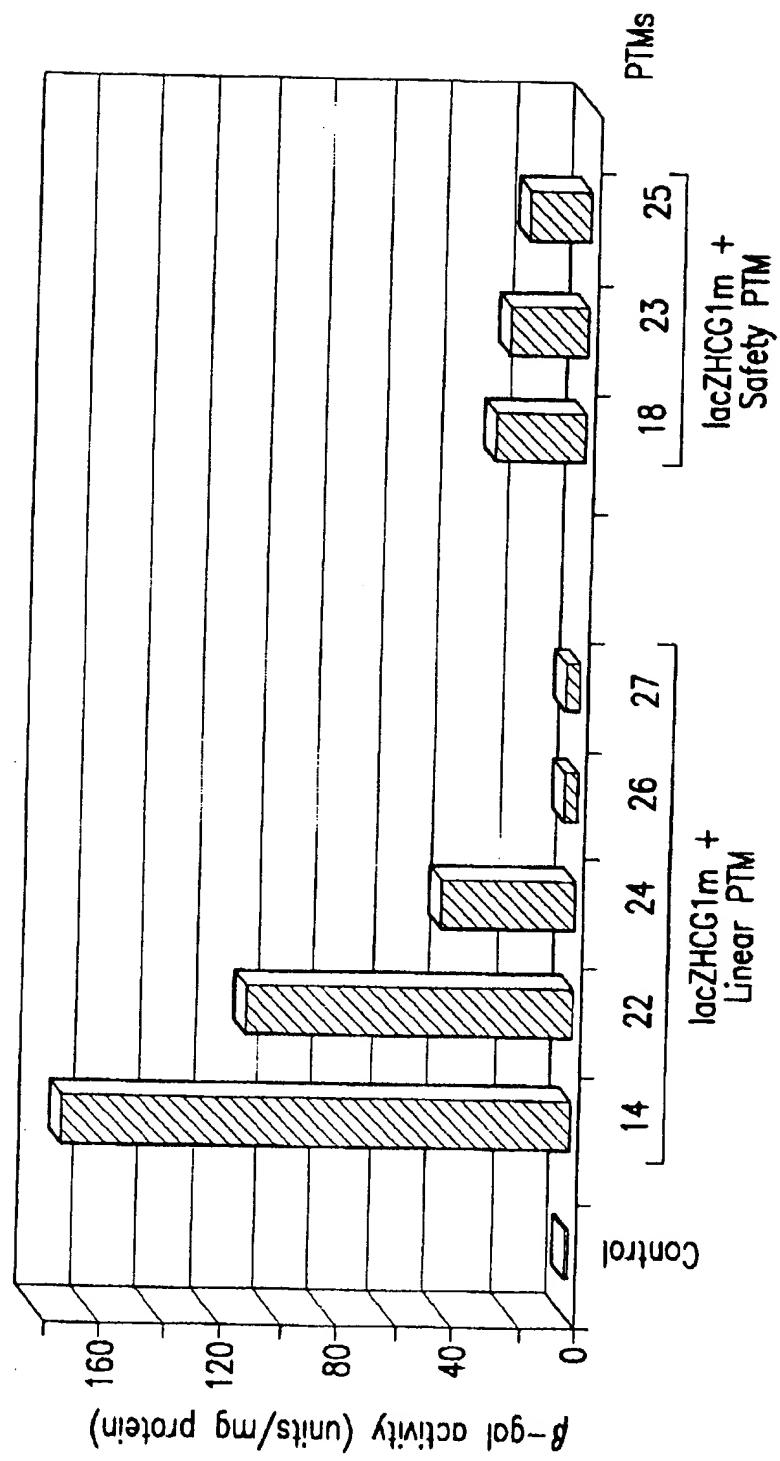


FIG. 41C

153 bp PTM24 Binding Domain:

Nhe I 153 bp BD underlined  
GCTAGC-AATAATGAGCGAGCCCTCAGGCTCAGGATTCACTGGCTCCAAATTATCATCCTAAGCAGAAGTGTATA  
TTCTTATTTGAAAGATTCTTAACTCATTGATTCAAAATATTAAAATACTTCCTGTTTACCTACTCTGCTATGC

Sac II  
AC-GCGCGG

FIG. 43A

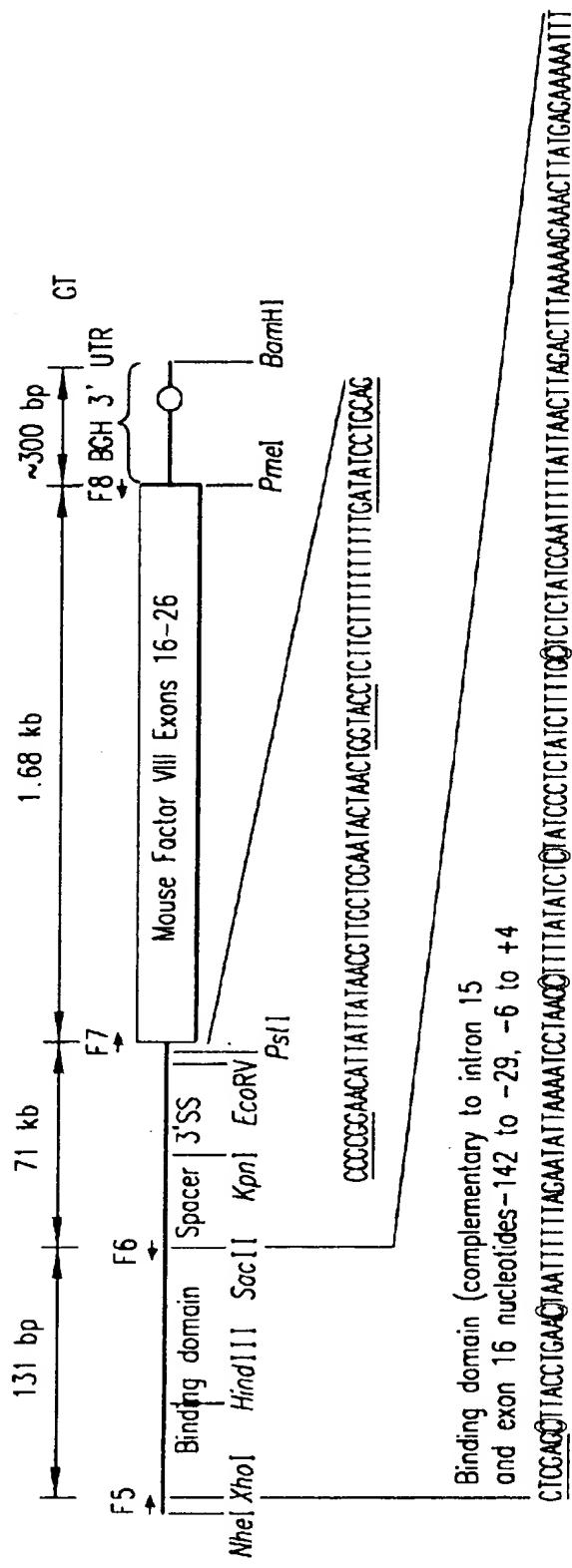


FIG. 44A

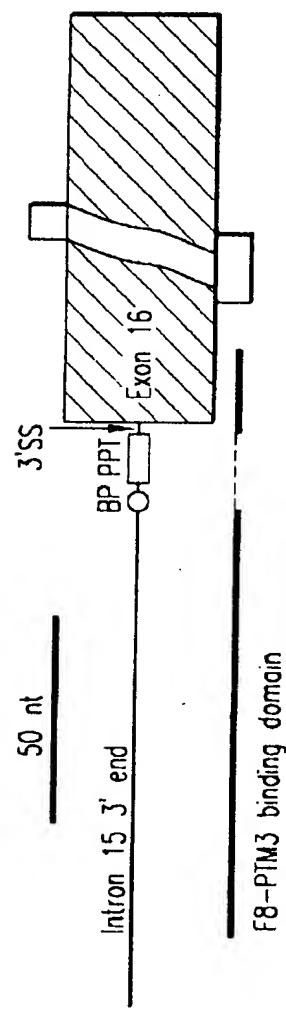


FIG. 44B

Sequence not included in construct

CGCCGCCCTCCCCCCCCCCCCCTCTGACTGACCCCGT TACTCCCAACGGTGA  
CGGGCGGGACGGGGCTCTCTCTCGGGCTGTAATTAGCGCTTGTTTAAATCAGGGCT  
TGTTTCTCTTCTGCTGCTGCTGAAGGCCCTGCTGAAGGGTCACTCGGGAGGAAATTCGTA

### Extent of promoter in phone construction

Extent of promoter in original construct

525 | 277 94 117

### Chicken Beta Actin Promoter (including exon 1 and part of intron 1)

FIG. 44C

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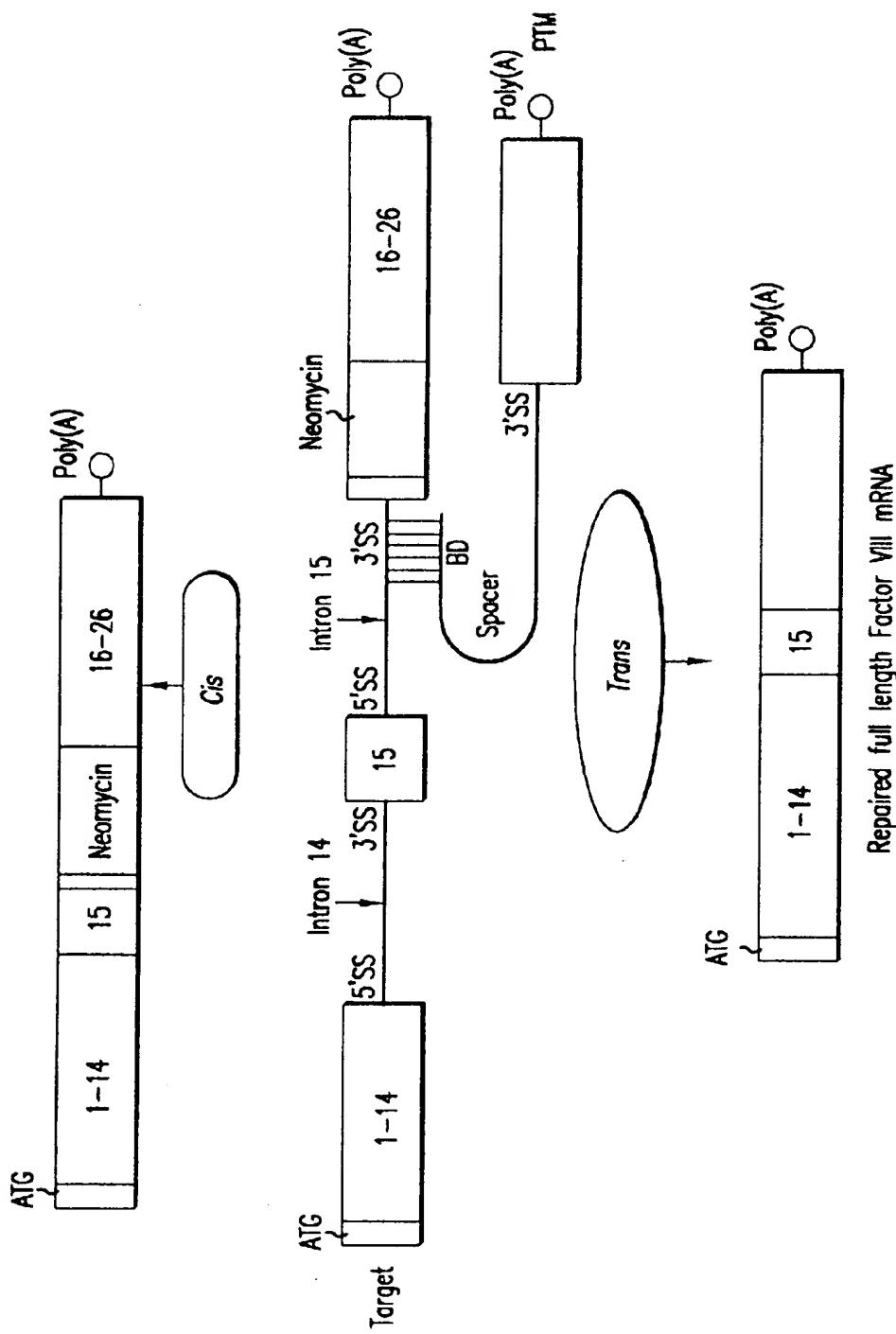
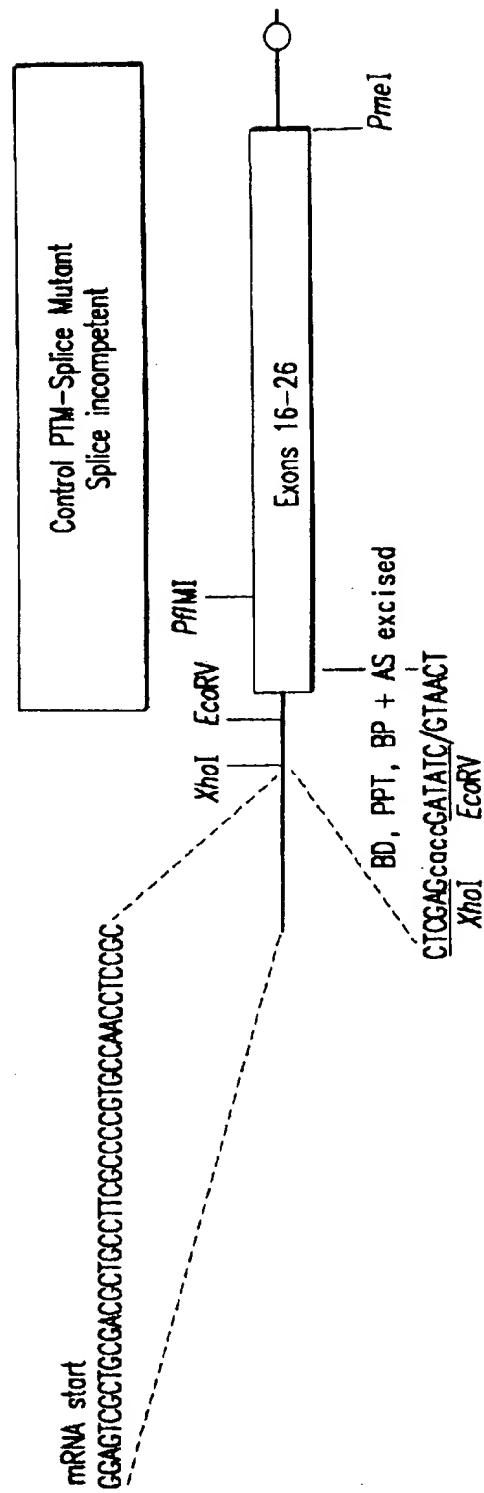


FIG. 44D

Repaired full length Factor VIII mRNA



**Method:**  
Excise TSD and part of exon 16 with  
XbaI and PPT1 and ligate in a PCR product that:  
1) eliminates the TSD and splice acceptor site  
2) inserts EcoRV adjacent to exon 16  
3) restores the coding for exon 16

FIG. 45

Repair of Factor VIII  
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice  
after IV PTM-FVII intraportal infusion  
(100 $\mu$ gDNA)(n=3)

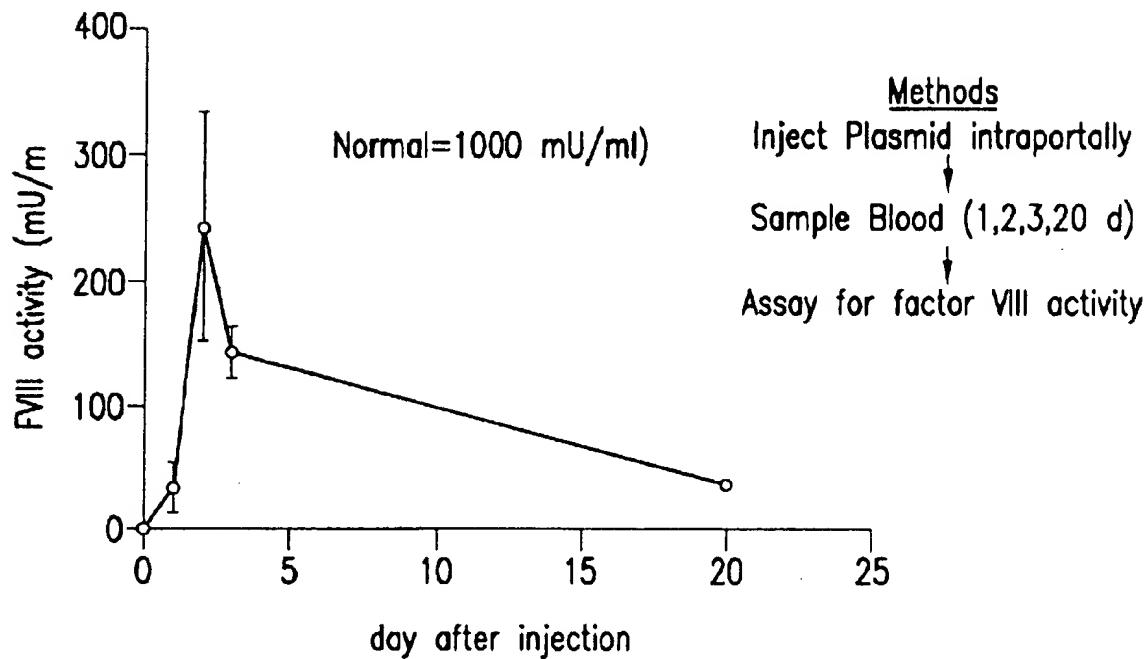


FIG.46

Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

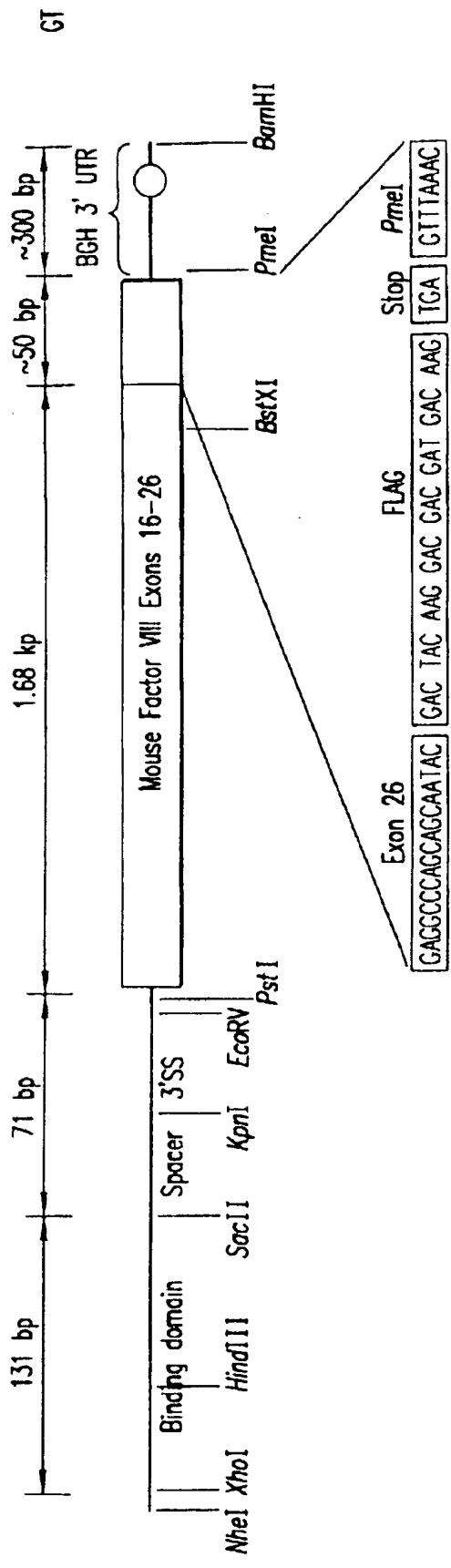
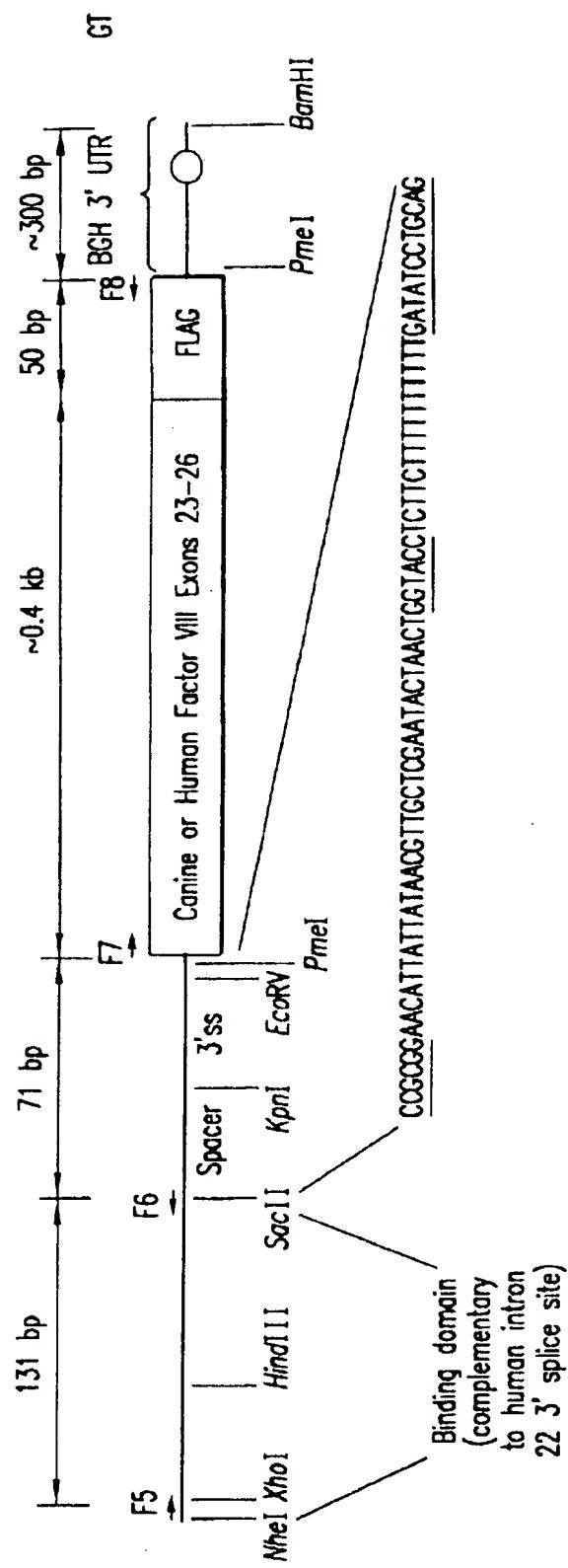


FIG. 47A



FLAG=C-terminal tag to be used to detect repaired factor VIII protein.

FIG. 47B